JAN 24 1931

Medical Library

PHYSIOLOGICAL REVIEWS

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

	PAGE
GAS TENSIONS IN THE TISSUES. *J. Argyll Campbell*	1
LOCAL REACTIONS IN SENSITIZED ANIMALS. ARTHUS PHENOMENON, "HYPERERGIC INFLAMMATION." *Martin Nordmann*	41
FACTORS IN THE DEVELOPMENT OF SEX AND SECONDARY SEXUAL CHARACTERISTICS. *Oscar Riddle*	63

~~This number contains Index for Volumes I to X~~

VOL. XI, No. 1

Issued January, 1931

BALTIMORE, U. S. A.

Entered as second-class matter, January 15, 1921, at the Post Office at Baltimore, Md., under the act of March 8, 1879

Made in United States of America

PHYSIOLOGICAL REVIEWS

PHYSIOLOGICAL REVIEWS is owned and controlled by the American Physiological Society. It is edited by a board appointed by the society consisting of:

W. H. HOWELL, Baltimore
LEO LOEB, St. Louis
J. J. R. MACLEOD, Aberdeen, Scotland
A. J. CARLSON, Chicago
P. A. SHAFFER, St. Louis
TORALD SOLLMANN, Cleveland
D. R. HOOKER, Managing Editor, Baltimore

The Editorial Board selects the subjects and authors of all articles which are published, the aim being to provide concise but comprehensive reviews of the recent literature and present status of various subjects in Physiology, using this term in a broad sense to include Bio-chemistry, Bio-physics, Experimental Pharmacology and Experimental Pathology. Each volume will consist of from sixteen to twenty articles, making a total of about five hundred pages per volume. The numbers will be issued quarterly in January, April, July and October of each year.

Subscriptions will be received in advance only. The subscription price is:

$6.00 per volume in the United States, net postpaid
$6.25 per volume in Canada, net postpaid
$6.50 per volume elsewhere, net postpaid
$2.50 per single number, net postpaid

Subscriptions should be sent to

D. R. HOOKER, *Managing Editor*
19 W. CHASE STREET, BALTIMORE, MD.

Contents of Volume X, 1930

M. L. ANSON AND A. E. MIRSKY: The Hemoglobin Compounds, with Special Reference to Protein Chemistry and the Significance of the Heme Pigments in Tissue Oxidations

J. H. BURN: The Errors of Biological Assay

H. E. MAGEE: The Physiology of the Small Intestines

E. D. ADRIAN: The Action of the Sense Organs

LEO LOEB: Individuality and Transplantation

V. E. HENDERSON: The Present Status of the Narcosis Theories

W. F. VON OETTINGEN: Excretion and Distribution of Bismuth Compounds

S. W. BRITTON: Adrenal Insufficiency

DAVID RAPPORT: The Interconversion of Foodstuffs

R. A. MCCANCE: The Chemistry of the Degradation of Protein Nitrogen

HERBERT S. GASSER: Contracture of Skeletal Muscle

HARDOLPH WASTENEYS AND HENRY BORSOOK: The Enzymatic Synthesis of Protein

A. C. IVY: The Rôle of Hormones in Digestion

PHYSIOLOGICAL REVIEWS

VOL. XI JANUARY, 1931 No. 1

GAS TENSIONS IN THE TISSUES

J. ARGYLL CAMPBELL

National Institute for Medical Research, Hampstead, London

Recent research on tissue gas tensions indicates that the oxygen tension is not nil in a normal resting animal, but positive (about 20 to 40 mm. Hg) in all regions examined, excluding the intestinal canal with food present and fermentation proceeding. A good deal of the older work was really in favour of such a conclusion, but it was passed over to make way for the evidence supporting the view that the tension was nil. The older estimations for oxygen tension in the blood were much at error, giving results much too low; it appears that this and Ehrlich's dye-reduction experiments were responsible for placing the oxygen tension in the tissues at or near nil. Although the figures for the blood have been corrected for many years, those for the tissues remained unaltered. It is now known that it is impossible, even by the most vigorous experimental procedure, to reduce the oxygen tension in the tissues in general to near nil, without risk of death.

DEFINITIONS. The gas tensions within the cell will be referred to as the intracellular gas tensions and those existing in the tissue fluids immediately outside the cell wall as the extracellular gas tensions. When gases pass from the capillary to the cell and *vice versa* they have to diffuse not only through the capillary endothelium, but also through a certain amount of tissue fluid or lymph before reaching the cell boundary. The advantage of using the term "extracellular" will be recognised when considering the method of determining tissue gas tensions by the injection of some gas, e.g., nitrogen, into the body cavities or under the skin; depots of gas are thus established and in these the tensions of oxygen and carbon dioxide after a certain time become constant and apparently similar to those existing in the fluids which moisten the surface of the serous membranes or cells under consideration; such tensions

are obviously extracellular, although they may resemble intracellular tensions. Under the heading gas tensions in the tissues there will be considered: *a*, intracellular and extracellular gas tensions; *b*, tensions in secretions and excretions, e.g., saliva, bile, urine, milk; *c*, tensions in the lumen of the alimentary canal; *d*, experimental alterations of tensions.

LAWS OF DIFFUSION. It is generally—but not always—admitted that the passage of gases through the tissues is regulated by the laws of diffusion. Graham's (1) law states that the rapidity of diffusion is directly proportional to the pressure and inversely proportional to the square root of the density of the gas. For diffusion through a liquid—the tissues are regarded as being more liquid than otherwise—Exner (2) found that the diffusion velocity is proportional to the coefficient of solubility of the gas in the fluid and inversely proportional to the square root of the density of the gas. In the tissues apart from the red corpuscles the gases are in solution; in other words, there is no great storage of any gas. If a gas is simply in solution and the temperature is constant the amount of it dissolved varies directly with the pressure (Henry's law, 3), so that if the pressure be doubled the solution will take up twice the quantity. The amount of gas dissolved in a fluid is proportional to the partial pressure of the gas. Dalton's (4) law of partial pressure states that in a mixture of gases each gas exerts the same pressure as it would exert if it were alone present in the volume occupied by the mixture. If several gases are brought together each of them will be distributed throughout the whole space as if the other gases were entirely absent. Partial pressure or tension of a gas must be distinguished from the quantity of the gas, the quantity being proportional to the tension. Thus if q equals the quantity dissolved in the fluid, k the coefficient, t the tension and p the atmospheric pressure, then $q = \frac{k \times t}{p}$, a simple formula in which tension is distinguished from quantity. The velocity of diffusion through the tissues depends upon the tension, and gases always diffuse from a region of higher tension to a region of lower tension.

GAS TENSIONS IN THE AIR AND IN THE BLOOD. The composition of atmospheric air at 50°N of the equator is given by Mellor (5) as carbon dioxide 0.02 per cent, oxygen 20.80 per cent, argon 0.94 per cent, nitrogen 77.32 per cent, and water 0.92 per cent, these figures being percentages of an atmosphere. Converting them into partial pressures we have approximately carbon dioxide almost nil, oxygen 158 mm. Hg, nitrogen and argon 595 mm., and water 7 mm., the total pressure at sea level being 760 mm. Hg. The above are the pressures existing in the

air as it is inspired. In the alveoli of the lungs the pressures are not the same as these. Haldane and Priestley (6) have found that the breathing is so regulated as to keep the partial pressures in the alveoli as follows: carbon dioxide 5.3 per cent (40 mm.), oxygen 14.0 per cent (106 mm.), nitrogen, argon, etc., 74.5 per cent (567 mm.) and water vapour 6.2 per cent (47 mm. Hg). The respiratory epithelium of the lung is exposed to these partial pressures, whilst in the venous blood circulating through the lung the oxygen tension or partial pressure is only 40 to 50 mm. Hg, so that oxygen will diffuse through from the alveoli into the blood, the head of pressure being 106 − 50 = 56 mm. Hg. In the venous blood the carbon dioxide tension is about 46 mm. Hg, that is 6 mm. higher than in the alveoli, so that this gas diffuses out from the blood into the alveoli, thus passing in the direction opposite to that for oxygen. When the blood leaves the lung it is arterial and contains oxygen at a partial pressure of about 100 mm. Hg and carbon dioxide at about 40 mm. Hg; at the arterial end of the lung capillary the oxygen tension in the blood has very nearly but not quite attained to the same value as that in the alveolar air (Bock and others (7)). The arterial blood passes to the tissues where it is converted into venous blood, because tissues use oxygen and therefore the tension of oxygen in the tissues is less than in the arterial blood and oxygen passes from the blood in the capillaries to the tissues; at the same time carbon dioxide is produced in the tissues and is at a higher tension there than in the arterial blood so that it diffuses out of the tissues into the blood. Summarizing, we have for oxygen tensions in external air 159 mm., in alveolar air 106 mm., in arterial blood 100 mm., in venous blood 40 to 50 mm., in extracellular fluids less than 40 to 50 mm., and inside the cell or intracellular similar to or less than the extracellular with a possible minimum at 0; oxygen therefore diffuses in the direction of the cells. For carbon dioxide tensions we have in external air almost nil, in alveolar air and arterial blood 40 mm. Hg, in venous blood 46 mm., in extracellular fluids and inside the cells greater than (or equal to) 46 mm., so that carbon dioxide diffuses in the direction away from the cells. The nitrogen tension throughout the blood and tissues is the same as in the alveolar air, namely, 75 per cent of the atmosphere.

From the above short description we see that whilst definite figures for gas tensions have been given for alveolar air and for blood, vaguer statements are made concerning the tissue gas tensions. Research in the latter direction has never been extensive because of the difficulties in approaching the problems; ideal technique is still wanting. Great

advance has been made in the past half-century in the study of the respiratory function of the lungs and of the blood. This knowledge has been of much value in the study of such problems as tissue gas tensions. Previous to these advances oxygen was supposed to be consumed by the blood which was also held responsible for the production of carbon dioxide. Pflüger (8) in 1872 thought that the tissues were the seat of combustion and rejected the theory of combustion in the blood. He and his colleagues tried to demonstrate that the tension of oxygen in the tissues was lower and that of carbon dioxide higher than elsewhere and although their results did give some such evidence, the technique and apparatus did not give very accurate results. Magnus (9) working in Heidenhain's laboratory was apparently the first to demonstrate that the venous blood coming to the lung contained less oxygen and more carbon dioxide than that passing from the lungs and thus disproved Lavoisier's contention that the lungs were the seat of combustion. More recent work as regards gas tensions in the blood and lungs centres round the researches of Haldane (10) on respiration and those of Barcroft (11) on blood.

DOES DIFFUSION SUFFICE? Before passing on to the tissues a brief reference to the question of diffusion through the lungs may be of advantage. Physiologists in general at the present day are agreed that diffusion suffices to explain the passage of gases through the respiratory epithelium although some years ago there was a good deal of discussion on the question (Bohr (12), A. and M. Krogh (13), Hartridge (14), Fredericq (15) Haldane (10) and others). The area of the respiratory epithelium is extensive, 100 sq. m., and the lung membrane is thin, 0.004 mm. in thickness; also its composition is mainly (90 per cent) water. Calculations, using the diffusion coefficients for such membranes, indicate that all the oxygen entering the blood in the lung in a given time either during rest or exercise, could pass through by diffusion alone. Loewy and Zuntz (16) calculated that a difference of partial pressure of oxygen less than 1 mm. Hg would account for the diffusion of 250 cc. of oxygen per minute through the alveolar walls. Haldane, Boycott and Damant (17) state that in the lungs the blood must become instantly saturated with nitrogen at the partial pressure existing in the alveolar air, so that when the pressure in the air is raised, as in diving, or lowered as at high altitudes, the change in the amount of nitrogen in the blood in the lungs must be instantaneous. Barcroft (18) discusses the problem of diffusion of oxygen through the lungs at high altitudes and gives much information in favour of the diffusion theory, showing that phenomena

of the type which it requires do really occur. How exactly the various factors concerned are changed in the lungs to meet the different conditions, e.g., rest, exercise, exposure to low oxygen pressure, has not been clearly worked out yet, but diffusion apparently suffices.

As regards diffusion of gases in the tissues, nearly all the work carried out recently has its origin in a paper by Krogh (19); in this he discussed the rates at which the oxygen molecules travel through the tissues, that is, the diffusion coefficients for oxygen in different tissues. He defines the diffusion constant as the quantity of gas diffusing through 1 sq. cm. surface and 1μ (0.001 mm.) thickness in 1 minute at a pressure difference of 1 atmosphere. He found that diffusion of gases through animal tissues is much slower than through water or gelatin and gives the following absolute diffusion constants for oxygen at 20°C.

Water	0.34	(after Hüfner)
Gelatin	0.28	(Krogh)
Muscle	0.14	(Krogh)
Connective tissue	0.115	(Krogh)
Chitin	0.013	(Krogh)
India rubber	0.077	(Krogh)

The constant increases with increasing temperature—about 1 per cent per degree taking the rate at 20° as unity. Using these constants and with the aid of Erlang's formula (20) it is possible to calculate the maximum tension difference, between that in the capillary and that in the tissue, necessary to supply the latter, e.g., muscle, with oxygen (see p. 24).

A. V. Hill (21) points out that in the tissues owing to the arrangement of the capillaries and the cells, we are dealing with very small distances, most conveniently measured in terms of μ, i.e., 1/1000 mm. and with times of very short duration conveniently measured in terms of σ, i.e., 1/1000 seconds. For systems involving such distances and times the diffusion constant is a fairly large quantity, being of the order of unity.[1] With the aid of Krogh's constants Hill studied the problem of diffusion of oxygen and lactic acid through tissues, giving mathematical solutions. He shows how a single resting nerve fibre 7μ thick would be adequately supplied with oxygen, when exposed to 0.0001 per cent of oxygen; in action the nerve would require just double this tension of oxygen. It is

[1] When expressed in units of centimeters and minutes, for aqueous solutions of ordinary substances K is usually of the order of 2 to 10 times 10^{-4}, which does not appear nearly so great.

difficult to asphyxiate a nerve fibre without absolutely pure nitrogen; on the other hand, the grey matter of the brain is particularly sensitive to low oxygen tension. Also the very rich blood supply in the interior of muscle is necessary partly because the fibres are larger and partly because of the relatively enormous heights to which oxygen requirements may rise during activity. In vigorous exercise there is an oxygen debt owing to the accumulation of lactic acid being more rapid than the diffusion of oxygen inwards. If there is no oxygen debt diffusion of oxygen is sufficient to keep the tension of oxygen positive everywhere. After a short burst of severe exercise, e.g., 200 yards' sprint by a 75 kilo man, the oxygen debt is about 10 litres and Hill calculates that this debt ought to be removed by diffusion of oxygen in about 8 minutes. Oxygen consumption estimations show that recovery, i.e., removal of oxygen debt, is 80 per cent or more complete in 8 minutes, an agreement which is close enough to the above calculated value. From this it would appear that the diffusion constants are fairly accurate and that diffusion suffices to explain the phenomena.

The changes in composition and rate of absorption of different gases injected into the pleural cavity were studied from a mathematical point of view by Rist and Strohl (22) using the laws and coefficients of diffusion; with relative units of time they built up curves which fit in fairly well with the results of actual experiment. Teschendorf (23) in his research upon the absorption of gases from the peritoneal and pleural cavities compared his observed rates of absorption with the Exner numbers at a temperature of 40°C. and found that an arrangement of the gases, in an order which takes into account the formula of Exner, approximates that made on the basis of the observed rates of absorption. McIver, Redfield and Benedict (24) in a study of the behaviour of carbon dioxide gas in the stomach of a cat, as a typical case of gaseous exchange, found strong evidence that the movement of this gas into and out of the stomach is not due to secretory factors, but is governed by the physical laws of diffusion. They give the curves of absorption and diffusion rates as calculated and as found; there is close agreement.

Secretion of oxygen. Most authorities are agreed that oxygen is not secreted by any tissue of man as it is in the swim-bladder of certain fishes. Haldane is the chief exponent of the secretion of oxygen by the lung under conditions of oxygen deficiency and his views will be found in his book on Respiration (10) and in a recent article in these Reviews (25). He holds that the wonderful efforts displayed by the climbers in the last Everest expedition could be explained only by secretion of

oxygen. At 28,000 feet the climbers suffered from excessive exhaustion and required eight or ten breaths for each step forward so that oxygen secretion was not helping them greatly at this altitude. In numerous experiments (26) on animals in which the oxygen tension in the tissues was estimated during prolonged exposure to low oxygen pressure in the inspired air there was no evidence that oxygen was being secreted by the lung to any advantage, since the tissue oxygen tension was remarkably low. Most observers have failed to confirm Haldane's views.

The production of oxygen by plants may be regarded as a secretion. In the animal kingdom the best example of oxygen secretion is that discovered by Biot (27) in certain Mediterranean sea fishes. In the swim-bladders of fishes caught at great depths there may be nearly pure oxygen, the amount of oxygen standing in close relationship with the hydrostatic pressure. Haldane (10) calculated that the oxygen tension on the inside wall of the swim-bladder was at least 1000 times greater than in the capillaries outside. Haldane gives a complete survey of this subject, describing how Moreau discovered the function of the swim-bladder and also Bohr's experiments proving that the secretion is under the control of the nervous system. There is no satsfactory evidence that the oxygen tension anywhere within the tissues of man exceeds that of the arterial blood, which is usually 100 mm. Hg. That in the tissues rarely reaches 60 mm. Hg even during hyperemia.

BIOGEN is mainly of historical interest and is the name which was given to a substance supposed to exist in the tissues and act as a store-house for oxygen. Biogen molecules were supposed to contain loosely combined oxygen in one side chain together with combustible substance in another, so that cell oxidations might proceed without immediate supply of oxygen. The theory was rejected some years ago, the evidence for and against it being summarized recently by Bayliss (28). The fact that carbon dioxide was given off by muscle in the absence of oxygen was interpreted as being due to a consumption of stored oxygen. It has been proved that this liberation is due entirely to lactic acid production (Fletcher and Brown, 29) and it is only in the presence of available oxygen that fresh production of carbon dioxide takes place. Any oxygen in the tissues is now considered to be either in stable combination or in solution, the amount in solution being small and depending upon the oxygen tension in the tissues.

LIFE WITHOUT OXYGEN. This subject has been reviewed from the physiological point of view by Bayliss (28) and by Clark (30). Certain bacteria are not only not killed by deprivation of oxygen, but cannot live

in its presence. In the case of intestinal worms, the leech and yeast cells, chemical activity of a special kind proceeds together with certain manifestations of life in the absence of oxygen. Intestinal worms are the only multicellular animals known which normally exist in the absence of oxygen. The glycogen in these animals is split into carbon dioxide, valerianic acid and hydrogen, the last being used up at once for reduction processes, that is, in a true fermentation. It is obvious then that life is possible where there is no tension of oxygen.

Clark, in his review, quotes Snyder who held that the beginnings of life on our planet were anaerobic. Our primitive atmosphere was devoid of free oxygen and the oxygen we now find is that liberated from carbon dioxide by the action of that green plant life which laid down enormous deposits of coal. This plant life was anaerobic and depended for its energy upon sunlight; but with the development of an oxygen atmosphere there arose organisms feeding basically upon plant life and taking advantage of energy derived by combustion. Clark states that we need not detract at all from the true importance of molecular oxygen in the economy of higher organisms, but he regards oxygen in a limited sense as a secondary invader, a scavenger clearing up the waste products of metabolism by combustion. If, for this process, the organism develops special mechanisms and seizes upon heat production, or upon special oxidation-reduction equilibria for special functions, so much the better. For higher organisms oxygen appears to be required at a definite tension in the immediate vicinity of the cell.

METHODS OF ESTIMATING GAS TENSIONS IN THE TISSUES. Ehrlich's (31) method of demonstrating the reducing power of the tissues by means of certain dyes was regarded for a long time as proving the existence or non-existence of oxygen at a positive tension. It is only recently that such phenomena have been properly investigated, and their biological significance has not been elucidated. Certainly the reduction of such dyes may have little to do with the presence or absence of free molecular oxygen. Statements that Ehrlich has proved the oxygen tension in the tissues to be nil are quite erroneous. The study of reduction of dyes has been much advanced by the recent work of Clark (32) and others on oxidation-reduction potential as measured in volts Eh, or as expressed in intensity of reduction $_{R}H$. A good deal of work has been done on simpler organisms, the $_{R}H$ of amoeba being given as 17 at pH 7.6 (33), that of eggs of echinoderms as 19 to 20 at pH 7.2 (33), whilst various figures, 10, 14 to 16, have been given for plant cells (34), (35). Fildes (36) concluded that the oxygen tension and the Eh of extracellular fluids

of the guinea pig were too high for germination of spores of *Bacillus tetanus*. Redslob and Reiss (37) found the $_{R}H$ of the vitreous of the rabbit's eye to be 19.1 at pH 7.6. These results of Redslob and Reiss may or may not correspond to an oxygen tension of about 40 mm. Hg; nothing definite is known as to such a relationship. Cannan (34) emphasizes the difficulty stating that there remains to be explained the occurrence within the same plant cell (*Mercurialis perennis*) of a photosynthetic production of oxygen under the pressure of the atmosphere and a state of reduction of the cell-pigment hermidin which can be in equilibrium with a pressure of only 10^{-60} atmospheres and that there probably exists in different parts of the same cell entirely different conditions. At the moment oxidation-reduction potential and oxygen tension are considered separately.

Four methods have been employed in the study of tissue gas tensions: A. Estimation of tensions from the amounts of gases in solution in the body fluids (Pflüger, 38, and others). B. Pflüger's aerotonometer method (Strassburg, 39) and its modifications (Krogh, 40). C. Verzar's (41) method. D. Estimations of gas tensions in depots of gas injected into the pleural cavity, the peritoneal cavity, the bladder, the lumen of the alimentary canal or into the subcutaneous tissue (Davy, 42; Tobiesen, 43; Pietro, 44; and others).

A. The tensions of gases determined from the amount of gas present in solution in fluids, secretions or excretions, removed from the body must be regarded with doubt because oxygen is used up in these fluids at a comparatively rapid rate. The gas pumps (Loewy, 45; Buckmaster and Gardner, 46) used in the extraction of the gases are not easy to manipulate and to keep air-tight. Such small amounts of oxygen are dissolved in body fluids that the entrance of even a small amount of air will vitiate the result. Then there is the necessity to assume that all the gas obtained was in solution.

B. In tonometer methods the fluid is removed from the body with exclusion of air; then a small bubble of gas is introduced and shaken up with the fluid; later the bubble is drawn off and the carbon dioxide and oxygen percentages estimated therein. This method often gives reliable results for carbon dioxide tension, but the oxygen tension found may be too low because oxygen may be used up in the fluids during the manipulations. In many of the earlier attempts with this method the amount of gas in the tonometer was too great and the length of time allowed for equilibrium much too short.

C. Verzar's method requires deep anesthesia and a good deal of dis-

section and manipulation of the tissue or organ under observation. Changes in oxygen consumption of the organ during anoxemia are estimated and also the oxygen saturation of the arterial and venous blood; from these data the oxygen tension may be calculated (see p. 24). Unless the experiment is of short duration anesthetics, e.g., urethane, amytal, will produce a lowering of oxygen tension (47) so that the results obtained would give lower limits only. The method failed in most cases, but reliable results seem to have been obtained with the submaxillary gland where a tension of about 40 mm. Hg. was found in the cell substance.

D. The method of injection—termed for convenience the injection method—of gas, usually nitrogen, into the body cavities or fluids or under the skin has given by far the most numerous and most constant results by different observers. With this method the oxygen tension is found to be positive wherever tested, ranging usually from about 20 to 40 mm. Hg for higher animals. With methods A and B described above the results of different workers varied from 0 to 200 mm. Hg. Observations with the injection method have been made in the pleural cavity, in the peritoneal cavity, in the bladder, in the washed-out stomach, in the washed-out intestine, in the subcutaneous tissue and in the body fluids of certain animals. There is a certain amount of criticism against the method. The presence of gas in the stomach and in the intestine cannot be called abnormal; but its presence in the other situations is certainly not normal; nevertheless the tensions for the alimentary canal are not very different from those for the other situations. The presence of gas in cellular tissues and in serous cavities does not lead to any serious symptoms or inconvenience to the subject. Injection of gas into the pleural cavity is a routine method of treating tuberculosis of the lung; injection of oxygen into the sheath of the nerve is a method of treating sciatica; in oxygen therapy for many purposes, oxygen has been injected under the skin of man, particularly in France (48); it has also been injected into joint cavities. When carried out with due care there is no extensive rupture of capillaries. In young animals, e.g., rabbits, cats, with loose skin the gas spreads out easily, but in animals with tight skins, e.g., guinea pig, monkey, there is greater damage to tissue. There can be no tearing of the tissue in the pleural, peritoneal and bladder cavities, nor in the lumen of the alimentary canal; nor in these regions does there appear to be any very significant reaction. I have observed frothy fluid in small quantities in the peritoneal cavity and also under the skin with the presence also of a fibrinous exudate;

the subcutaneous tissue tends to become more fibrous and dense in certain areas after numerous injections. There is no typical inflammation but there is some reaction.

Recently the reaction, under the skin of guinea pigs, to injection of oxygen, nitrogen, or carbon dioxide has been very carefully studied by Wright (49); as a rule he injected about 20 cc. every three days. The gross changes he describes include moistness or wetness of the tissue, sometimes injections of vessels, and an exudative fluid of greyish-white color. Microscopic changes consisted of formation of monocytes and modified monocytes from connective tissue cells at the end of two to four days. After three to four days some of these had become epithelioid cells, which after seven days had changed, sometimes into giant cells (Langhan's cell of tubercle). By eight or ten days the reaction which is of the sterile type, was at its height, with a maximum number of all the cells described above. There was also some exudate, whilst fibrin often covered the gas spaces as a thin membrane. Wolbach (50) had noticed such a fibrinous membrane lining spaces in emphysematous regions in the mediastinum. Wolbach did not mention the cellular exudates described by Wright, who concluded that gases injected into cellular tissue are capable of bringing about tissue changes which histologically resemble the anatomical tubercle; the reaction was found to differ only in degree from that which characterises tuberculosis, so far as cellular reaction is concerned. There were some few dead cells but no evidence of necrosis, nor of pus, nor of infection. When the latter conditions occur the oxygen tension falls to nil (see p. 20); clearly the reaction described does not resemble these conditions, since the oxygen tension is not nil, but 20 to 30 mm. Hg during Wright's reaction. He observed during the later states some dense fibrous tissue covered with flattened cells. Five years ago sections were cut and drawings were made for me of similar dense fibrous tissue with flattened epithelium from the subcutaneous tissue of a rabbit. The exudate was not examined and therefore its cellular contents were not noted. None of the tissue sections examined contained tubercles such as described by Wright; these may have been present in unexamined tissue.

Although all these reactions may proceed in the subcutaneous tissue, after the third or fourth day following the first injection the gas tensions in this region remain constant and do not change to any extent even though the injections be kept up for years. The same rabbits have been used often for long periods and for many different experiments, even when, under otherwise normal conditions, the carbon dioxide and oxygen

tensions remained the same as they were three or four days after the first injection. The curves give the changes in gas tensions following a single injection of air (fig. 1) and a single injection of nitrogen (fig. 2) under the skin of different rabbits. In the case of air the oxygen tension

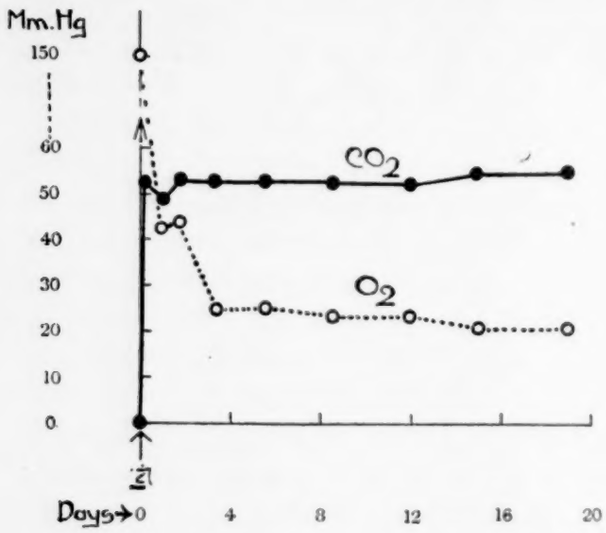

Fig. 1. CO_2- and O_2-tensions in gas under the skin (rabbit) for twenty days following the subcutaneous injection of atmospheric air. (Journ. Physiol.)

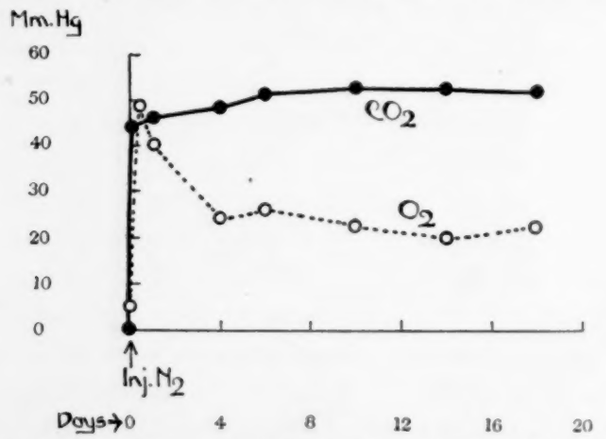

Fig. 2. CO_2- and O_2-tensions in gas under the skin (rabbit) for eighteen days after the subcutaneous injection of nitrogen. (Journ. Physiol.)

fell to the level at about 40 to 50 mm. Hg and remained there for about two days: in the experiment with nitrogen the oxygen tension rose to the level at about 40 to 50 mm. Hg and also remained there for about two days. In both cases after the third or fourth day the oxygen tension stood at about 20 to 30 mm. Hg and the carbon dioxide tension at about

50 mm. Hg at which they remained constant for the three weeks shown and for as long as the injections were kept up. Similar changes were observed if a mixture of gases with the tensions of oxygen at about 20 to 30 mm. Hg and carbon dioxide about 50 mm. Hg was injected (51). For the first two days following the first injection the oxygen tension rose to 40 to 50 mm. Hg and fell again later to 20 to 30 mm. Hg. This rise to 40 to 50 mm. Hg was considered to be due to hyperemia caused by the presence of the gas and passing off after the tissues had become more accustomed to it. Perhaps the formation of the slight exudate may have given some protection to the tissues from further irritation in this respect. A similar rise in oxygen tension during the first few days after the first injection was observed in the case of the peritoneal cavity (51). It could therefore not have been due to tearing of tissue. It is obvious that the changes described by Wright after the third or fourth day had no influence whatever upon the gas tensions; it is considered that these gas tensions indicate the tensions existing in the fluids moistening the cell walls of the subcutaneous tissue and of the peritoneal cavity and therefore probably existing in the cells themselves. That the reaction under the skin had an insignificant effect upon the tissue tensions was further confirmed by the observation that similar tensions have been observed in gas injected into the bladder (see p. 21) and into the lumen of the intestine (see p. 27). In some few cases after many injections of gas, with the gas remaining for long periods, there seemed to be an attempt on the part of the subcutaneous tissue to envelope the gas in places and hard knots were found containing a gas bubble surrounded by connective tissue. Wright obtained some evidence of formation of new elastic fibres. Even when the hard knots were formed there was no change in gas tensions. It is obvious from the curves in figures 1 and 2 that an excellent base line is obtained for experiments on changes in gas tensions within the body under various conditions.

When gas has been injected many times into the peritoneal cavity the frothy exudate which covers the intestine in small quantity, contains some lymphocytes both small and large, mononuclear cells (apparently derived from the endothelium), polymorphonuclear leucocytes and some endothelial cells; apart from a reaction with evidence of some proliferation there does not appear to be any adverse change in the peritoneal tissue itself nor is there any evidence of interference with the normal functions of the animals, which ate their food readily, moved about actively and put on weight as usual. Further the gas tensions remained constant over long periods, years, as long as the animals were under observation.

A great advantage of this method of injection of gas under the skin and into the peritoneal cavity is that no anesthetic is required and there is little interference with the animal; injections into the pleural cavity interfere with the movement of the lung. When injecting under the skin of an animal, it is best to use the skin of the back. In all cases large vessels must be avoided during introduction of the needle. Samples are withdrawn, with careful exclusion of air, and analysed in the Haldane or other apparatus.

When gas is injected into the tissues its rate of absorption depends upon its solubility and its rate of diffusion (22), (24); carbon dioxide is absorbed most readily, 500 cc. and over disappearing within a few hours after injection under the skin of the back of a rabbit (51) or into the pleural cavity of a man (52), (43). Nitrogen disappears very slowly, some of it remaining for three or more weeks after injection of large quantities, 500 cc. or more, under the skin of a rabbit (fig. 2). Air is nearly as slow (fig. 1). Oxygen is absorbed more rapidly than either air or nitrogen, but more slowly than carbon dioxide (51); after injection of large quantities under the rabbit's skin some of it remains for at least a week. There is some evidence (53) that gases are removed more readily from the abdominal cavity than from under the skin. Nitrogen and air are used for inducing artificial pneumothorax because of their slow rate of removal (54).

Haldane (10) states that gas free in the tissues or as a bubble in blood tends eventually to disappear because the partial pressure of nitrogen in the free gas becomes greater than that in solution in the blood, which is saturated in the lungs with nitrogen at a pressure of about 75 per cent of the atmosphere. The total pressure of gases in solution in the venous blood is only about 94 per cent of an atmosphere, i.e., 75 per cent nitrogen, 6 per cent oxygen, 6.5 per cent carbon dioxide and 6.5 per cent water vapour; on the other hand the total gas pressure in the free gas is that of the atmosphere so that its nitrogen pressure is about 6 per cent above that of the venous blood, the other factors being much as before. The nitrogen in the free gas goes slowly into solution in the blood and is removed by way of the lungs.

NORMAL GAS TENSION IN THE SUBCUTANEOUS AND OTHER CELLULAR TISSUES. As early as 150 years ago air and other gases were injected under the skin to determine whether gas was the cause of typical inflammation; the results were negative (Astley Cooper, quoted by Abernethy, 55; Monro and Bell, quoted by Leconte and Demarquay, 56; Beddoes, quoted by Demarquay, 57). Davy (42) in 1823 appears to have been

the first to study the composition of gases in cellular tissue; he obtained the gas from the mediastinum of a patient who had died from dysentery. Leconte and Demarquay (58) in 1863 analysed gases from a case of emphysema following fracture of a rib and found in the cellular tissue oxygen 6 per cent (42 mm. Hg) and carbon dioxide 5 per cent (35 mm.) figures which appear to be accurate. They (56) record a large number of analyses which they carried out on rabbits injecting air or oxygen; it is impossible to draw conclusions from these results since the figures for carbon dioxide were obviously inaccurate in many cases. They quote experiments by Bouley and Clement, the results of which seem fairly accurate as far as they go; in 48 hours after injection, the oxygen tension was 4.39 per cent (31 mm. Hg) and the carbon dioxide 8.29 per cent (58 mm.). Sertoli (59) records results for one short experiment in

TABLE 1

Homoiothermous animals: subcutaneous gas tensions, mm. Hg

ANIMAL	CO_2	O_2
Rabbit	49	23
Cat	43	22
Rat	52	24
Guinea pig	52	20
Hedgehog	51	27
Fowl	46	25
Monkey	49	40
Man	40	43

which air was injected, the carbon dioxide tension being 6.52 per cent (46 mm. Hg). Plumier (60), in experiments lasting for 1 hour up to some days, found an oxygen tension varying from 6 to 8 per cent (42 to 56 mm. Hg). Most of the earlier results were obviously inaccurate being handicapped by technique, and it is quite impossible to bring them all into line. Recent and numerous experiments (61) with different animals have given constant results, sufficient time being allowed for the true constant level to be attained (table 1). For homoiothermous animals the carbon dioxide tension lies usually between 45 to 55 mm. Hg and the oxygen tension between 20 to 30 mm. Hg. In man and monkey the oxygen tension is higher than in other animals; in the case of man the gas was injected under the skin of the forearm. Large numbers of observations were made on different rabbits during the past six years and the percentage distribution within limits of 5 mm. Hg for

150 of the observations is given elsewhere (61). Different animals of the same species sometimes showed fairly marked differences in normal tensions, but in any one animal the tensions remained constant under normal conditions.

Bazett and Sribyatta (62) carried out experiments upon man following the changes only during the first hour or so after the injection of small quantities of gas so that their results should require some slight modification before comparison with those in the table; nevertheless their results are similar.

In calculating the tensions, water vapor in the tissues is taken as exerting a tension of 47 mm. Hg. The remainder, after deducting the tensions for carbon dioxide, oxygen and water vapor, is regarded as being due to nitrogen and traces of other gases; traces of a combustible gas which had apparently come from the intestine were (63) sometimes

TABLE 2

Poikilothermous animals: subcutaneous gas tensions, mm. Hg

ANIMAL	AIR TEMPERATURE	CO_2	O_2
	°C.		
Toad	3	4	100
Toad	17	12	49
Frog	17	14	48
Toad	23	14	63
Snake	15	21	24
Tortoise	15	23	20

found. The average barometric pressure where the observations were made was about 747 to 750 mm. Hg and the air temperature about 15 to 20°C.

Some results (61) were obtained for poikilothermous animals (see table 2). The tensions under the skin of frogs and toads depend upon the temperature, the carbon dioxide being lower at 3°C. than at 17° whilst the oxygen tension is much higher at the former temperature. The carbon dioxide tension of 14 mm. at 17°C. agrees very well with the figure given by Krogh (64) for carbon dioxide tension in frog's blood; he has proved that frogs use the skin as a respiratory organ. In the tracheal system of the hind legs of a grasshopper he found 16 per cent of oxygen during rest and only 5 per cent (about 37 mm. Hg) during exhausting muscular exertions (65). The oxygen tensions in the covering tissues surrounding the tracheal system must be lower than these.

The percentages of carbon dioxide were considerably lower than the oxygen deficit and a large part of the carbon dioxide formed in the tissues must be carried away by other means than through the tracheae.

A number of observations have been carried out on other lower animals by Fredericq (15), Adler (66) and Gaarder (67) the last two using the injection method. Fredericq studied the tensions in the nourishing fluids and blood of animals living in sea water, e.g., crustaceans, worms, sea urchins, and found the carbon dioxide tensions to be less than 1 per cent of an atmosphere whilst the oxygen tensions ranged from about 1 up to 14 per cent. There is, however, an objection to his results since the fluids were removed from the animals and these fluids use up oxygen. Adler injected gas into the general body cavity of the worm in small quantities and under the surface covering or chitin of some *Tracheata*; after allowing a certain time to elapse he analysed the gas in Krogh's microapparatus. In the general body cavity of the worm, *Lumbricus terrestris*, he found an average oxygen tension of 1.9 per cent of an atmosphere. His average results for *Tracheata* are given in percentages in table 3, for ordinary conditions of surrounding atmosphere; broadly speaking, these figures represent from 90 to 130 mm. Hg.

TABLE 3

Tracheata; O_2-tension under chitin; per cent atmosphere

Cossus ligniperda (caterpillar)	12.8
Leucorrhinia (larva)	2–16
Pieris brassicae (chrysalid)	12.6
Sphinx ligustri (chrysalid)	18.8
Tenebrio molitor (larva)	14.1
Tenebrio molitor (nympha)	14.1

The variations were insignificant except in the case of Leucorrhinia. Gaarder (67) estimated the oxygen tension under the chitin on the dorsal aspect of *Tenebrio* pupa and found figures (12.9 to 16.1 per cent) similar to those of Adler. Gaarder noted some connection between the variations in the metabolism and the oxygen tension in the tissues; an increase in metabolism caused a slight fall in oxygen tension and *vice versa*.

NORMAL GAS TENSIONS IN THE PERITONEAL CAVITY. Monro (56) is mentioned as being the first, in 1771, to inject gas into different body cavities. Astley Cooper (55) proved that air injected into the abdominal cavity was harmless, being absorbed and disappearing completely,

no inflammation resulting; these experiments were concerned with the idea that air might be a cause of putrefaction of living tissue. Recently as we have seen it has been found that injection of gas does cause some reaction slight in degree compared with typical inflammation.

In 1859 Leconte and Demarquay (56) studied the changes in composition of gases injected into the peritoneal cavity of rabbits using air, oxygen, carbon dioxide, nitrogen and hydrogen. They observed that with air or oxygen, the oxygen diminished progressively whilst carbon dioxide increased until it formed about 5 per cent. As there were errors in their technique no general conclusion can be drawn from the results. Sertoli (59) also used rabbits following the changes for two hours only; his results appear to be fairly accurate for carbon dioxide, 4.7 to 7.2 per cent.

Pietro (44) in 1902 carried out the first series of accurate observations using dogs chiefly, and following the changes up to seven days. He

TABLE 4

Tensions in peritoneal cavity, mm. Hg

ANIMAL	CO_2	O_2
Rabbit	47	37
Cat	41	29
Rat	52	33
Guinea pig	56	28
Monkey	49	40

injected either oxygen, nitrogen, hydrogen or carbon dioxide alone. The carbon dioxide was absorbed in a few hours so that he was not able to follow out these changes for long. With the other three gases he found that the final oxygen tension was always about 5 to 7 per cent, as was also the carbon dioxide tension. When hydrogen was the gas injected all the hydrogen had disappeared by the 3rd day, its place being taken by carbon dioxide, oxygen and nitrogen.

Haggard and Henderson (68) used dogs, injecting either air or nitrogen, and concluded that the carbon dioxide tension of the abdominal gas soon becomes equivalent (37 to 40 mm. Hg) to that of the pulmonary alveolar air and of the arterial blood; this, however, holds only for the earlier stages following the injection (69). In one case they followed the oxygen tension changes for 54 hours and concluded that the oxygen tension in the peritoneal cavity was about 45 mm. Hg and that this was the tension actually existing in the cells. They, however, did not follow

the changes long enough to determine the tension at the level of constancy. This has been done only recently (51). When this level has been attained the tension remains constant under normal conditions as long as the animals have been under observation. Tensions for different kinds of animals have been studied (61) and some of the average results are given in table 4. Pietro (44) gave 36 and 39 mm. Hg for the oxygen tension of dogs which agree very closely with the figures for the monkey, rabbit and rat.

Hydrogen is found in the peritoneal cavity of the rabbit, cat and monkey, having diffused through from the lumen of the intestine by way of blood and lymph, and arising from fermentation of the food since it disappears on starvation (63). The tension of hydrogen in the peritoneal cavity is usually about 7 mm. Hg, but it has been found to reach 21 mm. In a monkey the following tensions were noted: hydrogen 21 mm. Hg, carbon dioxide 46 mm., oxygen 43 mm., water vapour 47 mm., and the remainder 603 mm. apparently nitrogen chiefly with traces of argon.

Some observations were carried out in which nitrogen was injected into the abdominal cavity of frogs kept at 17°C. The tension of carbon dioxide was 14 mm. Hg agreeing with the figure given for frog's blood by Krogh (64); the oxygen tension in the abdominal cavity was about 28 mm. Hg.

GAS TENSIONS IN THE PLEURAL CAVITY. Astley Cooper (55) over 130 years ago injected air into the pleural cavity and demonstrated that it did not cause inflammation. Many others, including Wintrich (70) carried out similar observations. Wintrich employed calves, dogs, cats and rabbits, injecting nitrogen, oxygen, carbon dioxide, and sulphuretted hydrogen; he found that none of these gases except the last mentioned produced typical inflammation. Recent research indicates that there will be some slight reaction with any gas.

Much attention was directed to the presence of gas in the pleural cavity because this occurs sometimes spontaneously with tuberculosis of the lung; Von Itard (see Tobiesen, 52) in 1805 called this condition pneumothorax. Further, between 1888–1892 Forlanini, according to Hamman and Sloan (71), instituted injection of gas into one pleural cavity as a therapeutic measure for the same malady.

John Davy (42) in 1823 analysed gases obtained from the pleural cavity. His analyses did not give accurate results because they were made *post mortem* when the carbon dioxide tension is greatly increased and the oxygen tension decreased. Many of the earlier observers, e.g.,

de Boismont (72), Solon (73), Demarquay and Leconte (58), Ewald (74) and Hoppe Seyler (75) studied pathological cases where the pleura was affected and found high figures, over 10 per cent, for carbon dioxide tension and very low figures, almost nil, for oxygen tension. This apparently occurs anywhere when the tissues become infected; I found a very low oxygen tension, 2 to 3 mm. Hg, in subcutaneous cellulitis. Other observers have recorded gas tensions for the pleural cavity under conditions which were obviously fairly normal since their figures agreed very well with those given for normal conditions in other regions. Among these researchers are included Webb, Gilbert James and Havens (76), Tobiesen (43), Tachau and Thilenius (77), Rist and Strohl (22), Dautrebande and Spehl (78), and Grass and Meiners (79), (80). Some

TABLE 5

Gas tensions in pleural cavity, mm. Hg

AUTHOR	SUBJECT	CONDITION	GAS INJECTED	CO_2	O_2
Webb and others	Monkey	Normal	Air	56	30
Webb and others	Monkey	Normal	Nitrogen	60	39
Tobiesen	Man	T.B. lung, no pleurisy	Nitrogen	40	27
Tobiesen	Man	T.B. lung, no pleurisy	Air	44	24
Tobiesen	Man	T.B. lung, no pleurisy	Oxygen	43	34
Tobiesen	Man	T.B. lung, no pleurisy	Carbon dioxide	42	38
Grass	Man	T.B. lung, no pleurisy	—	46	37
Grass and Meiners	Man	T.B. lung, no pleurisy	—	47	38
Grass	Man	Pneumothorax with pathological changes	—	95	0
Grass	Man	Pneumothorax with pathological changes	—	89	7

of their results for practically physiological conditions are shown in table 5. The percentages found are given in the original papers but I have converted them into tensions, mm. Hg; they resemble fairly closely those in table 4. The figures in table 5 for carbon dioxide obtained by Webb and others look somewhat high, but this is due to the fact that the monkeys were killed with chloroform before the gas samples were withdrawn. After stoppage of the circulation carbon dioxide accumulates very rapidly in the tissues and the oxygen tends to disappear more slowly (68), (81). The results in table 5 have been specially selected because it is obvious from all the results examined that the greater the infective process, e.g., thickening or necrosis, in the pleural membrane the higher does the carbon dioxide tension rise and the lower does the oxygen tension fall; a few results of Grass for such pathological

conditions are given at the end of table 5 illustrating this point (see also 82).

Tobiesen's results for the pleural cavity indicate that the type of gas injected does not influence to any extent the final result. This agrees with Pietro's (44) observation for the peritoneal cavity and with other results from subcutaneous injections (51).

NORMAL GAS TENSIONS IN SECRETIONS AND EXCRETIONS. *Urine.* In the earlier researches (Planer, 83; Schöffer, 84; Morin, 85; Pflüger, 38; Ewald, 86) attempts were made to estimate the tension of gases in urine by extracting the gases by vacuum pump or by boiling, or by shaking out with inert gases; tensions were calculated from the amounts of gases obtained which were regarded as being merely in solution. In the gas pump observations the oxygen tension was found to be almost anything from nil up to 200 mm. Hg. This throws doubt upon the accuracy of the technique. Strassburg (39) used Pflüger's aerotonometer, whilst Fredericq (15) used Krogh's more accurate modification.

TABLE 6

Gas tensions in normal urine (man), mm. Hg

CO_2	O_2	OBSERVER
—	20–35	Krogh
40–50	14–35	Campbe l
45	50–60	Buckmaster and Hickman
—	42–95	Mainzer

More recent observers (Krogh, 87; Buckmaster and Hickman, 88; Campbell, 89, and Mainzer, 90), also using one or other modification of the tonometer method have found a positive oxygen tension in urine (see table 6). They show variations, but Mainzer's higher figures require confirmation, his method being rather longer and more complicated than that of the others. In support of the other figures it may be stated that gas tensions in the bladder cavity (89) are much the same. Mixtures of gases were injected by way of the urethra into the emptied bladders of normal rabbits and a monkey; at intervals up to three hours samples were withdrawn, the carbon dioxide tension in 17 observations varying between 37 and 63 mm. Hg whilst the oxygen tension varied between 33 and 61 mm. Hg. The monkey's bladder figures were 47 for carbon dioxide tension and 42 for oxygen tension, much the same as were obtained for its subcutaneous tissue tensions and for those in its peritoneal cavity. The disadvantage of estimating tensions in the urine

voided from the body is that oxygen is used up by urine (Berthelot, 91). Krogh (87) found the tension of oxygen to be 35 mm. Hg in a sample of urine and the tension of this sample did not change over a considerable period. The same figure was obtained for urine rapidly formed by the kidney and excreted in a marked diuresis (89).

Urine under pathological conditions may contain other gases, namely, hydrogen, methane and sulphuretted hydrogen, the first two being formed by fermentation in diabetic urine (Heyse, 92; Senator, 93) and the last mentioned originating from protein in cystitis (Rosenheim, 94). These conditions are not physiological and will not be considered further.

Lymph. All the older researches were handicapped by technical difficulties. Many of them (Dähnhardt, 95; Hensen, 96; Hammarsten, 97; Tschirew, 98; Buchner, 99; Gaule, 100) estimated the quantity-volume per cent of carbon dioxide removable by gas pump and by chemical means, usually finding less than in venous blood. Gaule considered the tension to be higher than in venous blood, whilst Strassburg (39) found the tension to be intermediate between that of arterial and venous blood. Some carbon dioxide may have been lost during the collection of the lymph. Some of Strassburg's results suggest that the tension was at least about 42 mm. Hg. Most of these observers found a certain amount of oxygen indicating a considerable tension in some cases, but they regarded this oxygen as having been drawn into the pump by accident from the air. Their figures for oxygen tension in blood were so low (and so much at error) that there was no other view to take regarding the presence of oxygen in secretions.

Ewald (101) examined samples of tissue fluids, e.g., from subcutaneous edema, pleuritic effusion; Planer (83) gives some figures for peritoneal effusion and Strassburg (39) some for fluid from a case of hydrocele. These are pathological conditions and the carbon dioxide tension was often high, e.g., about 80 mm. Hg (Ewald). Pembrey (102) has reviewed this subject giving fuller details.

The figures given already for normal tensions, i.e., oxygen 20 to 40 mm. Hg, carbon dioxide 40 to 50, in the pleural and peritoneal cavities and under the skin are probably the same as exist in normal lymph, since serous cavities contain a certain amount of serous fluid; also in the subcutaneous tissue a certain amount of exudate was observed to cover the tissues and to be in contact with the injected gas (see p. 11).

Saliva. Pflüger (38) examined the gases in a number of secretions and excretions, viz., urine, milk, bile and saliva, finding the following aver-

ages for nitrogen 0.90, 0.75, 0.50 and 0.75 volume per cent respectively, and for oxygen 0.07, 0.09, 0.10 and 0.50 respectively. The value 0.50 volume per cent for oxygen in saliva is very high; if the oxygen is considered as being in solution it represents a tension of over 150 mm. Hg. Külz (103) repeated these experiments, obtaining even higher figures which Barcroft (104) modified by deducting a certain quantity regarded as being due to air drawn into the pump; with this correction Külz' figures resembled those of Pflüger. Nevertheless, these very high tensions cannot be accepted without further confirmation. Fredericq (15) using Krogh's aerotonometer found about 7 mm. Hg tension for oxygen and for carbon dioxide about 49 to 92 mm. Hg; oxygen may have been used up during these estimations. Verzar (41) estimated the oxygen tension (intracellular) in the cells of the submaxillary gland to be about 40 mm. Hg and this figure seems to be the most accurate of all those under consideration. It agrees well with tensions obtained for other regions of the body.

Bile and milk. In bile Fredericq (15) found the carbon dioxide for various animals to be as follows: dog 9 per cent, goose 9.6 per cent, ox 9.6 per cent, calf 9.3 per cent, pig 9.5 per cent and for the invertebrate *Torpedo marmorata*, 0.0 per cent. Strassburg (39) also obtained about 7 to 9 per cent for the dog's bile so that for mammals the carbon dioxide tension is probably about 49 to 63 mm. Hg. Many of the older workers (see Pembrey, 102) and more recently Buckmaster and Hickman (88) estimated the volume per cent of gases removable from bile. Most of them appear to have obtained oxygen in solution indicating a positive oxygen tension. Oxygen may be used up in bile and it is not possible to draw any conclusions regarding its tension therein; further investigations are necessary.

The amounts of gases removable from milk (human, cow's, goat's) have also been investigated (Setchenow, 105; Pflüger, 38; Thörner, 106; Hoppe-Seyler and Külz, 107) and although nothing very definite can be concluded from the results as a whole, the oxygen tension always appeared to be positive.

NORMAL GAS TENSIONS IN MUSCLE. Verzar (41) attempted to estimate the oxygen tension in resting muscle of a cat. His argument was as follows: If oxygen comes into the tissues by diffusion, then the quantity, Q, going into the tissue depends on the difference of oxygen-pressure in the blood, P, and in the tissues, p, other things being equal and $Q = d(P - p)$. If now as has been supposed $p = o$, then if P decreases Q must decrease in the same way and $Q = dP$. The oxygen consumption,

therefore, if the oxygen pressure is diminished in the blood, must diminish in the same rate. If this is not the case it must be concluded that p (the pressure in the tissues under normal conditions) is not nil, but has a positive value which is capable of reduction if P falls. He estimated the amount of oxygen used by the muscle and the oxygen pressure in the blood traversing it, i.e., in the blood entering and leaving it. He determined how the oxygen consumption Q changed when the oxygen pressure P was changed by breathing air with lowered oxygen pressure. With muscle he found a diminution in oxygen use on lowering the oxygen pressure in the air breathed and from his calculations obtained as maxima for oxygen tensions in four experiments the figures 24.5, 29.5, 41.5 and 19 mm. Hg respectively, and concluded that the tension in normal resting muscle must be below 19 and even nil; but his muscle was not normal; all the veins were tied except one and the tissue had been much handled and dissected; also urethane was used as the anesthetic with A.C.E. mixture and urethane lowers oxygen tension in the tissues. Verzar's conclusions for muscle cannot be accepted; yet many have restated his conclusion that the tension of oxygen in resting muscle is nil. If it were nil, then lactic acid would accumulate, but Simpson and Macleod (108) and others have shown that lactic acid is present only in minute quantities in resting muscle, if proper technique is used for its extraction; from this it appears that oxygen tension is positive everywhere in resting muscle. Indeed, Krogh (20) concluded that the oxygen tension in the muscle must be equal to that of the venous or capillary blood because of the very rich capillary supply even when all the capillaries were not open. In a later paper he (109) describes a method of injecting capillaries with india ink which enabled him to make counts of the numbers of open capillaries. It seems curious that his countings should have led him to conclude in this paper that the oxygen tension is, sometimes at least, very low in resting muscle, but that in working muscle it approaches very near to that of the blood, because it is in working muscle that we find the most lactic acid. Possibly the oxygen tension may be positive in certain areas within the muscle fibre whilst lactic acid is present in too great a quantity in others to be immediately removed. Krogh's method of estimating the number of open capillaries in resting muscle may, of course, not be strictly accurate. Barcroft (18) in discussing muscular fatigue as the cause of mountain sickness states that so great are the possibilities of increased capillary supply that the actual asphyxiation of the muscle substance is not a very serious factor in ordinary exercise at high altitudes and therefore the

effects of altitude must for the most part be sought elsewhere. He also indicates that the oxygen tension in venous blood will still be about 10 mm. Hg even where the alveolar oxygen pressure has fallen to 52 mm. and the oxygen consumption has increased to threefold its normal value. It is, therefore, not an easy matter to reduce the oxygen tension in the tissues to anywhere near nil in a living mammal and this has been proved recently by experiment (see p. 33).

Henderson (110) considered that the oxygen tension of the venous blood leaving an active muscle would be about 20 mm. Hg. He does not seem able to decide definitely what the oxygen tension in the cell might be and gives two sets of figures and curves, one to cover an oxygen tension in the tissues of 20 mm. Hg, and the other an oxygen tension of nil. During heavy work Krogh (109) and Lindhard (111) found the oxygen tension in venous blood to be only about 12 mm. Hg so that the oxygen tension in the working muscle would be of a similar low order. Taking all the evidence into consideration, it is concluded that in normal resting voluntary muscle, the oxygen tension is positive and probably about 40 mm. Hg.

It is not known what the oxygen tension is in the heart muscle fibre or in the plain muscle fibre. Although the frog's heart can continue to function in an atmosphere of nitrogen for many hours, the mammalian heart ceases to beat when its coronary circulation is restricted and it is thus deprived of its oxygen supply (112). When a mammal is exposed to low oxygen-pressure in the inspired air the heart becomes dilated and its muscle is one of the tissues affected by prolonged exposure to such conditions exhibiting pathological changes (26). With regard to plain muscle we know that oxygen is necessary for maintenance of tonus (113).

Gaarder (114) studied the oxygen consumption in the fish *Cyprinus carpio* under various pressures of oxygen in the water and concluded that the oxygen tension in a part of its tissues is nil under normal conditions. He used urethane which may have influenced the result. Of course, among some of the lower members of the animal kingdom, life without oxygen becomes a reality, so that the oxygen tension in their substance must be nil.

Gases in the alimentary canal. Each part of the alimentary canal is exposed to gases with composition more or less characteristic. Thus, whilst the mouth, esophagus and stomach often contain some pure air, the large gut contains little oxygen, a relatively small proportion of nitrogen and much carbon dioxide, methane and hydrogen.

It is possible to estimate the carbon dioxide tension in the mucous membrane of the mouth by enclosing some air between the cheek and gums; in about 30 to 60 minutes the carbon dioxide tension is about 5 to 7 per cent (35–49 mm. Hg). J. Davy (42) in 1823 studied the effects of a mucous membrane upon air enclosed in a cavity. He punctured the maxillary antrum and frontal sinus of a sheep after death and collected the imprisoned air; he found about 4.5 per cent of carbon dioxide.

If, without interference with the circulation, gas be imprisoned in any part of the empty alimentary canal after careful washing out with saline at 37°C., the tensions of the gases in the lining mucous membrane may be studied. Normally the composition of the gases within the lumen often depends to a large extent upon the changes in the food it contains and this composition does not give the tensions in the mucous membrane although such composition is influenced by diffusion through this membrane.

TABLE 7

Gas tensions in the stomach, mm. Hg

SUBJECT	CO_2	O_2	AUTHOR
Dog	30–40	—	Schierbeck
Man	31–34	—	Ylppö
Man	30–60	—	Dunn and Thompson
Cat	39–52	10	Edkins
Cat	35–47	10	McIver, Redfield and Benedict
Cat	40–51	20	Campbell (recent observations)

Stomach. Gas tensions in the mucous membrane have been estimated by injecting gas (or sometimes water) into the emptied and washed out stomach (Schierbeck, 115; Ylppö, 116; Edkins, 117; Dunn and Thompson, 118; McIver, Redfield and Benedict, 24). Some of the results are recorded in table 7. In the longer experiments, up to about three hours, the carbon dioxide tension was usually about 40 to 50 mm. Hg. The oxygen tension appears to be at least about 20 mm. Hg. Deep anesthesia with amytal or urethane lowers the oxygen tension in the tissues at least about 7 mm. Hg, so that Edkins' and McIver's results are probably too low. Dunn and Thompson found the carbon dioxide tension in some normal men to be 60 mm. Hg and they fix this figure as the normal maximum. The tensions in the table are extracellular and probably also intracellular. Schierbeck thought that

carbon dioxide was secreted by the stomach and found much higher values when food was present. Dunn and Thompson and also Edkins could not confirm this effect of food; nor has secretion been confirmed. Schierbeck's technique is open to criticism; he estimated the tension from the amount in solution in water injected into the stomach.

Under normal conditions small amounts of air are swallowed with the food and saliva; the composition of the air is soon changed because carbon dioxide diffuses out from the mucous membrane of the stomach whilst oxygen diffuses in the opposite direction. In abnormal conditions, e.g., dyspepsia, large quantities of air may be swallowed. The reason for this is not known, but it recalls air swallowing by certain fish, e.g., *Cobitis fossilis* (Erman, 119; Leydig, 120) and *Callichthys asper* (Jobert, 121), which will live without water. The oxygen of the air swallowed diffuses through the mucous membrane of the alimentary canal which is very vascular, the remains of the air and the carbon dioxide being discharged per rectum. It is possible that in dyspepsias in human beings, air is swallowed to supply oxygen to an abnormal mucous membrane. Ylppö calculated that about 12 cc. of oxygen may pass through the normal stomach wall per minute from air in the cavity.

In pathological conditions in the human stomach where the food is retained for long periods owing to some blockage at the pylorus, the food undergoes fermentation by bacteria and yeasts with the formation of hydrogen, methane and sulphuretted hydrogen. The literature on this subject is extensive and some reference to it will be found in papers by Popoff (122), Kuhn (123), Hoppe-Seyler (124) and Kantor (125). Although fermentation of food in the stomach is abnormal for omnivora and carnivora it is quite physiological for herbivora. Thus, whilst Planer (126) found only oxygen, nitrogen and carbon dioxide in the stomach of normal dogs fed on different diets, Tappeiner (127), (128) obtained hydrogen in the stomach of the horse but no methane. In the paunch of oxen, cows, goats, sheep and lambs both these gases were present. In pigs, Tappeiner (129) found some hydrogen when the diet was either cabbage or peas or rye; and methane was present in addition when on a cabbage diet. In ruminants methane may form 30 per cent, hydrogen 5 per cent, and carbon dioxide 60 per cent of the gas normally present in the paunch; small amounts of nitrogen and very small amounts of oxygen are then present.

GASES IN THE INTESTINE. Gas tensions in the mucous membrane of the cleaned out small intestine have been estimated using the injection of gas, by McIver and others (24); some results are given in table 8.

In all the recent results the oxygen tension was proved to be positive. It is advisable to inject a mixture of gases with oxygen varying between 3 and 5 per cent and to leave the gas in the intestine for several hours with the abdomen stitched up and the body of the animal under saline at 37°C. The tensions given are extracellular but probably exist within the cells lining the mucous membrane.

The gases found in the intestine with its normal contents present include oxygen, carbon dioxide, nitrogen, hydrogen, methane, sulphuretted hydrogen and methylmercaptan. They arise partly from gases passed in from the stomach, partly by diffusion through the mucous membrane and mainly from fermentation. Gases do not diffuse into the completely emptied intestine but when fluid is present the fluid contains gases in solution (126) which have come from the mucous membrane. Carbon dioxide arises also from cleavage of carbonates, lactates, acetates and citrates, from alcoholic fermentation of glucose (130),

TABLE 8

Gas tensions mm. Hg in small intestine (mucous membrane)

SUBJECT	CO_2	O_2	AUTHOR
Dog	49–63	—	Strassburg
Cat	35–47	40	McIver and others
Cat	45	35	Campbell (recent observations)

from putrefaction of carbohydrates, e.g., cellulose, from putrefaction of proteins and from butyric and lactic acid fermentations. Hydrogen is also formed in these fermentations. Protein putrefaction gives origin also to sulphuretted hydrogen and methylmercaptan. Methane is developed from decomposition of acetates, lactates, lecithin of cholin (131), (132) and in particular from cellulose decomposition. A complete review of the subject of cellulose fermentation has been published recently by Marjorie Stephenson (133); the reader is referred to her article for the literature and also for details regarding the enzymes and bacteria concerned. Macfayden, Nencki and Sieber (134) and also Moore and Rockwood (135) studied the changes in the small intestine and concluded that under normal conditions fermentation occurs only to a slight extent therein, the large intestine being the chief site of gas formation.

In man, observations on gas content of different regions of the ali-

mentary canal were carried out by Magendie and Chrevereul in 1816 (136), by Planer in 1860 (126) and by Tappeiner in 1886 (137) on the bodies of criminals after death. The tensions given in table 9 are only approximate and have been calculated from some of Tappeiner's results after allowing 47 mm. Hg for vapour tension. The carbon dioxide tension in the stomach is about twice as high as it is in the living man because soon after death this gas accumulates rapidly in the body. A much better indication of the gases produced in the large intestine in living man is obtained from the analysis of gases passed per rectum (Planer, 126, Ruge, 138, Schmidt, 139, and Fries, 140). Tensions calcu-

TABLE 9

Post-mortem gas tensions in the alimentary canal of man, mm. Hg

GAS	STOMACH	ILEUM	COLON	RECTUM
Oxygen	65	—	—	—
Nitrogen	529	482	53	447
Hydrogen	0	28	3	—
Methane	1	—	—	6
Carbon dioxide	116	202	655	259

TABLE 10

Tensions of gases freed from large intestine of living man, mm. Hg

GAS	MILK DIET		MEAT DIET			VEGETABLE DIET		
	48 hours	72 hours	24 hours	48 hours	72 hours	48 hours	72 hours	96 hours
Carbon dioxide	120	64	97	89	60	242	274	150
Methane	6	0	266	197	188	318	352	398
Hydrogen	313	387	21	15	5	17	11	29
Nitrogen	274	262	328	413	459	136	76	135

lated from some of Ruge's results with different diets are given in table 10. An interesting point about these results is the apparent absence of oxygen so that oxygen tension must be nearly nil and at the most only traces of oxygen have been recorded in this region. Any oxygen diffusing through from the mucous membrane would disappear in the fermentation process in which hydrogen is liberated. A fair amount of oxygen may diffuse through and be used up in this way.

The times given in table 10 refer to the durations on the special diets. There seems to be a high proportion of hydrogen formed on a milk diet and of methane when either on a vegetable or a flesh diet. Schmidt's

results, however, showed a high proportion of hydrogen also on a diet of legumes. He states that this is so because carbon dioxide and methane diffuse more rapidly through the mucous membrane into the blood vessels and escape *via* the lungs.

Most of the work connected with gas in the intestine has been carried out on animals. Tappeiner (127), (128), (129) gives large numbers of results for dogs, geese, pigs, oxen, goats and horses. Usually the animals were fed for a week or two on a fixed diet, the gases then being obtained shortly after death; Planer (126), Ruge (138) and Hofmann (141) give similar results. The general conclusion from all these results is that methane is found in the intestine of herbivora and omnivora, but not in that of carnivora. Hoffmann did not detect any methane in rabbits, but Tappeiner (142) did when the diet was of pease meal. There were also in most animals high figures for carbon dioxide and lower figures for hydrogen; broadly speaking, the results on a vegetable diet for gases in man's large intestine in table 10 suffice also as examples for most of the animals mentioned above.

It may be concluded that when food is present in the alimentary canal the oxygen tension is practically nil in all parts except the stomach, whilst carbon dioxide, hydrogen or methane may exist at very high tensions (50 per cent of an atmosphere) in the large intestine.

These very high tensions of these gases lead to their absorption in part and excretion by way of the lungs. Hydrogen has been detected in the tissues (63). Bunge (143) estimates that 6 litres of carbon dioxide are liberated by hydrochloric, lactic and butyric acids daily, by neutralisation of the acid chyme by sodium carbonate of the intestinal juice; carbon dioxide is readily absorbed and excreted by the lungs (144), (145) whilst hydrogen with a lower coefficient of absorption is liable to produce distention. Fries (140) stated that about 1 litre of gas is freed daily per rectum by man. On the other hand, the carbon dioxide produced in the alimentary canal in a grown up steer forms 6 to 8 per cent of the total carbon metabolised; 100 to 250 litres of methane are formed daily in the gut of cattle. Zuntz and others (146) found in rabbits that most of the hydrogen and methane were freed by way of the lungs[2] whilst in the horse most of the methane was freed per rectum; in the latter there were about 74 litres of carbon dioxide excreted otherwise than by the lungs, 13 by the gut and about 61 by the skin in 24 hours. Planer (126) ligatured the rectum of a dog, shutting in the feces for about seven days;

[2] Very recently Parsons proved that traces of hydrogen and methane are present in the expired air of man. (Journ. Biochem., xxiv, 585, 1930.)

the animal kept healthy and took some food. There was only a temporary accumulation of gas—as might be expected in a carnivorous animal—which disappeared owing to diffusion of gases into the circulation. He found in the stomach about 39 per cent of carbon dioxide, 6.5 per cent of hydrogen and a trace of sulphuretted hydrogen. Flint (147) and Claude Bernard (148) found that sulphuretted hydrogen injected into the rectum was absorbed and excreted by the lungs, but less rapidly than when injected into a vein.

The tension of nitrogen in the alimentary canal varies and where there is fermentation rarely approaches that of the tissues which is about 75 per cent of the atmosphere. The production of carbonic acid, hydrogen and methane in the large intestine is great and the nitrogen diffuses out slowly from the mucous membrane, so that its tension remains low. Krogh (149) states that under normal conditions no free nitrogen is generated by fermentation. Protein metabolism does not give rise to any excretion of free gaseous nitrogen from the body. Oppenheimer (150) claims that denitrifying bacteria are present in the intestine of herbivora and capable of splitting off nitrogen from nitrite and nitrates when these are present.

EXPERIMENTAL ALTERATIONS IN TISSUE GAS TENSIONS. The curves shown in figures 1 and 2 (see p. 12) indicate that the injection method supplies an excellent base line for experimental purposes. By injecting 200 to 300 cc. of nitrogen once a week into the abdominal cavity and under the skin long experiments have been carried out recently. It is rather surprising that this advantage of the method was not discovered sooner.

The effects of prolonged exposures of animals to altered oxygen pressure in the inspired air are shown in figures 3 and 4; the former gives the effects upon carbon dioxide tensions in two regions and the latter the effects upon the oxygen tension in the peritoneal cavity. From these results it was concluded that even after considerable acclimatisation to low oxygen pressure in the inspired air, the tissue oxygen tension remained low (151), so that secretion of oxygen by the lung, if it did exist, was not helping the animal to any extent. In addition it was evident that the tissue carbon dioxide tension did not play any great part in the ability to tolerate low oxygen pressure in the inspired air, because although in cats and rabbits the carbon dioxide tension fell to a similar low level, the cats did not tolerate the very low oxygen pressure becoming collapsed, yet the rabbits were very resistant.

It was also demonstrated in experiments each covering about a week

that carbon monoxide poisoning lowers the oxygen tension in the tissues to a more marked extent than does a similar loss of function of hemo-

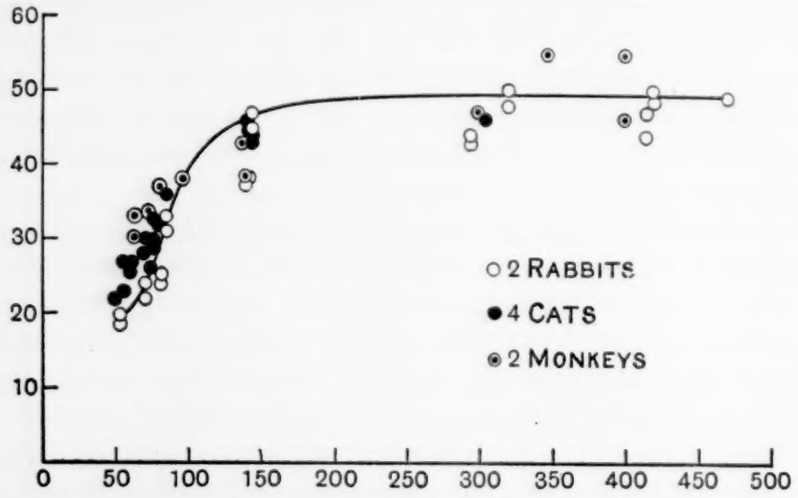

Fig. 3. Relations between CO_2-tensions in the tissues and O_2-pressure in the inspired air during prolonged exposures. The curve is drawn through points taken from a rabbit. (Journ. Physiol.)

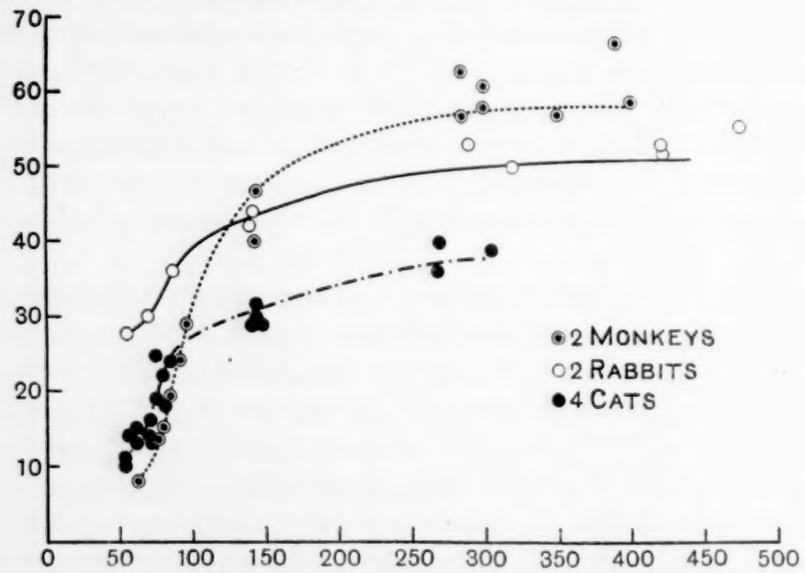

Fig. 4. Relations between O_2-tensions in the abdominal cavity and O_2-pressure in the inspired air during prolonged exposures. (Journ. Physiol.)

globin by hemolysis and bleeding, the same rabbits being used for both series of experiments (152). The carbon monoxide tension in the tissues was estimated by similar technique in these experiments and was

found to be much lower than that in the inspired air; because of the low oxygen tension in the tissues, the hemoglobin does not part so easily with the carbon monoxide.

In some other experiments (153) the hemoglobin content of the blood was increased by as much as was possible, e.g., 60 per cent, by injections of blood into rabbits or by previous prolonged—4 weeks or more—exposure to low oxygen pressure in the inspired air. Increase of oxygen content of blood in this way leads to an increase by 25 per cent and more, in oxygen tension in the tissues.

Oxygen want produced by hemolysis (153), bleeding, breathing oxygen at low pressure, etc., lowers the oxygen tension in the tissues to much the same extent in each case, although bleeding seems to be somewhat less effective than hemolysis or exposure to low oxygen pressure in the air. These experiments proved that in the living animal it is not possible, even by extreme procedures, e.g., loss of 70 per cent of hemoglobin, to lower the oxygen tension in the tissues below 40 per cent of the normal value for any length of time without risk of death. It is absurd, therefore, to state that under normal conditions the oxygen tension in the tissues is nil.

Following muscular exercise or convulsions the oxygen tension in the tissues is increased to 25 per cent above normal, probably because change in pH in the tissues affects the dissociation of oxygen from the blood (69). In many types of tetany (154) a fall of oxygen tension in the tissues was observed before the onset of the tetanic contractions or convulsions (table 11). It is suggested that tetany is caused by a lack of oxygen in the nerve cell of the brain or spinal cord and that convulsions are of value in that they are followed by a rise of oxygen tension in the tissues. In one case following vigorous exercise the oxygen tension under the skin reached 70 mm. Hg; this is the highest figure I have found for oxygen tension within the tissues whilst breathing oxygen at normal pressure; it was due in part to previous hyperemia (160).

Injection of acid-producing substances also causes a rise of oxygen tension in the tissues and a fall of carbon dioxide tension (68), (69). On the other hand, alkalosis produced by hyperpnea or by sodium bicarbonate causes a fall of oxygen tension in the tissues and evidence of tetany. In table 11 the oxygen deficiency theory of tetany is compared with other well-known theories and appears to be of more universal application, if there is one and only one cause of tetany; this, of course, may not be the case.

The effect of local temperature changes upon gas tensions in the sub-

cutaneous region has been studied by Bazett and Sribyatta (62) who found that under the skin of the forearm the carbon dioxide tension increased as the temperature increased from 10° to 50°C.; so did the oxygen tension within the limits 22 to 40°C.; below 22°C. there was a rise in the oxygen curve due, they thought, to a decrease in metabolism. However increase of metabolism in rabbits by thyroid feeding did not produce any appreciable change in gas tensions in the tissues (53).

As regards general temperature changes, Schott (155) found an increase in oxygen tension and a fall of carbon dioxide tension under the skin of rabbits as the effect of certain saline baths at about 80°F.

TABLE 11

Table showing comparison of theories

EXPERIMENTAL CONDITION	FALL OF O_2-TENSION IN TISSUES BEFORE ONSET OF MANIFEST TETANY OR CONVULSIONS	ALKALOSIS	GUANIDINE POISONING	CALCIUM DEFICIENCY
Excessive hyperpnea	+	+		
Guanidine injection	+		+	
$NaHCO_3$ injection	+	+		
Na_2SO_4 injection	+			±
NaCl injection	+			±
Insulin injection	+			
Histamine injection	+			
Alcohol injection	+			
Water injection	+			
Ammonium salts injection	+	±		
Anoxemia	+			
Cyanide injection				
Strychnine injection				
Parathyroidectomy		∓	∓	+

Exposure of the whole body of a rabbit to a carbon arc lamp, sufficient to cause a rise in rectal temperature of 1.5°C. definitely raised the oxygen tension in the subcutaneous tissue and lowered the carbon dioxide tension (69). These effects may have been due to dilatation of vessels. Stimulation of the heart by caffeine produced similar and more marked effects (69).

The effects of vaso-constrictor substances, e.g., pituitary extract and adrenaline chloride, in reducing the oxygen tension in the tissues and increasing the carbon dioxide tension are easily demonstrated by the injection method (51). The action is more marked under the skin than

in the abdominal cavity. In one case the oxygen tension in the subcutaneous tissue of a rabbit was reduced from 28 to about 4 mm. Hg by a large dose of pituitary extract (156).

With a poisonous dose of histamine (ergamine acid phosphate) the oxygen tension was reduced from 14 to 2 mm. Hg and the carbon dioxide tension increased from 46 to 108 mm. Hg in the subcutaneous tissue of a cat; the animal was collapsed and in a state of shock but recovered the next day (51).

The changes in oxygen and carbon dioxide tensions in the tissues produced by changes in the pressure of oxygen and carbon dioxide in the inspired air have been studied. Breathing oxygen at several atmospheres' pressure causes profound increase in oxygen tension and increase to a dangerous level in the carbon dioxide tension in the tissues (157); the oxygen tension lies much above the curve shown in figure 4. Lowering of the barometric pressure produces a marked decrease in oxygen and carbon dioxide tensions similar to that produced by breathing oxygen at low pressure in the air (158). Breathing carbon dioxide at high percentage, e.g., 30, does not affect the oxygen tension appreciably, but markedly increases the carbon dioxide tension in the tissues which approximates eventually to that in the inspired air (157), (159). On the other hand, marked hyperpnea greatly lowers the carbon dioxide tension; this fell from 52 to 8 mm. in the abdominal cavity in one case (160) and from 50 to 21 mm. in the subcutaneous region in another (161).

In whatever way the tissue gas tensions be altered by experiment, when the animal settles down again to normal resting conditions, the carbon dioxide and the oxygen tension in the tissues return to their original normal levels. There is a normal tissue oxygen tension and if this be altered the organism, as soon as possible, endeavours to restore it to the normal level again. For all mammals tested, including monkey and man, the normal tissue oxygen tensions have been found to lie between 20 and 40 mm. Hg. It is suggested that in the geological epoch when tissues were first shut off from the external atmosphere or sea, such tensions (20 to 40 mm. Hg) of oxygen existed in this medium; a similar hypothesis is advanced for carbon dioxide tensions in the tissues, the normal figures for which are usually about 40 to 50 mm. Hg.

Throughout all the regions studied in the higher animals at rest, there is only one situation where the oxygen tension approaches nil; this is in the intestinal canal with food present and fermentation proceeding.

It is of interest in connection with the oxygen tension (40 mm. Hg)

which has been found to exist in many mammalian tissues, to recall that Pantin (162) recently observed a similar oxygen tension (30 to 40) in the surrounding medium to be the critical tension for amoeboid movement (marine amoeba); below this tension the activity decreases. He refers to the literature on this point.

BIBLIOGRAPHY

(1) Graham, T. Elements of chemistry, 1842, 71, 72.
(2) Exner, F. Ann. d. Phys. u. Chem., 1875, clv, 321, 443.
(3) Henry, W. Phil. Trans. Roy. Soc., 1803, xxix, 29, 274.
(4) Dalton, J. Mem. Manchester Lit. Phil. Soc., (2), 1805, i, 271.
(5) Mellor, J. W. A comprehensive treatment of inorganic and theoretical chemistry, 1928, viii, 5.
(6) Haldane, J. S. and J. G. Priestley. Journ. Physiol., 1905, xxxii, 225.
(7) Bock, A. V., D. B. Dill, H. T. Edwards, L. J. Henderson and J. H. Talbott. Journ. Physiol., 1929, lxviii, 277.
(8) Pflüger, E. Pflüger's Arch., 1872, vi, 43; 1875, x, 251.
(9) Magnus, G. Ann. d. Phys. u. Chem., 1837, xl, 583; 1845, lxvi, 177.
(10) Haldane, J. S. Respiration, 1922, Yale Univ. Press.
(11) Barcroft, J. The respiratory function of the blood. Pt. II. Haemoglobin, 1928, Cambridge Univ. Press.
(12) Bohr, C. Skand. Arch. f. Physiol., 1909, xxii, 221.
(13) Krogh, A. and M. Krogh. Skand, Arch. f. Physiol., 1910, xxiii, 179, 248.
(14) Hartridge, H. Journ. Physiol., 1912, xlv, 170.
(15) Fredericq, L. Arch. Intern. de Physiol., 1911, x, 391; Arch. de Biol., 1896, xiv, 105.
(16) Loewy, A. and N. Zuntz. Die Physiologischen Grundlagen der Sauerstoff-Therapie, in Michaelis' Die Sauerstofftherapie, Berlin, 22, 1906.
(17) Haldane, J. S., A. E. Boycott and Damant. Journ. Hygiene, 1908, viii, 342.
(18) Barcroft, J. The respiratory function of the blood. Pt. I. Lessons from high altitudes. 1925, Cambridge Univ. Press.
(19) Krogh, A. Journ. Physiol., 1919, lii, 391.
(20) Krogh, A. Journ. Physiol., 1919, lii, 409.
(21) Hill, A. V. Proc. Roy. Soc., 1928, civ, 39.
(22) Rist, E. and A. Strohl. Ann. de Med., 1920, viii, 233.
(23) Teschendorf, W. Arch. f. exp. Path. u. Pharm., 1924, civ, 352.
(24) McIver, M. A., A. C. Redfield and E. B. Benedict. Amer. Journ. Physiol., 1926, lxxvi, 92.
(25) Haldane, J. S. Physiol. Reviews, 1927, vii, 363.
(26) Campbell, J. A. Lancet, 1928, ii, 84.
(27) Biot. Mem. d. l. Soc. d'Arcueil, 1807, 254.
(28) Bayliss, L. E. Principles of general physiology (2nd ed. revised) 1918.
(29) Fletcher, W. M. and G. M. Brown. Journ. Physiol., 1914, xlviii, 177.
(30) Clark, W. M. Journ. Wash. Acad. Sci., 1924, xiv, 123.
(31) Ehrlich, P. Das Sauerstoff-Bedürfniss des Organismus, 1885, Berlin.

(32) Clark, W. M. et al. Hyg. Lab. Bull., Treas. Dept. U. S. Public Health Service, 1928, cli.
(33) Needham, J. and D. M. Needham. . Proc. Roy. Soc., B. 1925, xcviii, 259.
(34) Cannan, R. K. Biochem. Journ., 1926, xx, 927.
(35) Rapkine, L. and R. Wurmser. C. R. Soc. Biol., 1926, xciv, 1347; Proc. Roy. Soc. B. 1927, cii, 127.
(36) Fildes, P. Brit. Journ. Exp. Path., 1929, x, 197.
(37) Redslob, E. and P. Reiss. C. R. Soc. de Biol., 1929, cii, 1060.
(38) Pflüger, E. Pflüger's Arch., 1869, ii, 156.
(39) Strassburg, G. Pflüger's Arch., 1872, vi, 65.
(40) Krogh, A. Skand. Arch. f. Physiol., 1908, xx, 259, 279.
(41) Verzar, F. Journ. Physiol., 1912, xlv, 39.
(42) Davy, J. Phil. Trans. Roy. Soc., 1823, cxiii, 496.
(43) Tobiesen, F. Deutsch. Arch. klin. Med., 1914, cxv, 399.
(44) Pietro, S. di. Arch. ital. de Biologie, 1902, xxxviii, 102.
(45) Loewy, A. In C. Neuberg's book, Der Harn sowie die ubrigen Ausscheidungen und Körperflüssigkeiten von Mensch und Tier. Tiel. ii, 1911, 1287.
(46) Buckmaster, G. A. and J. A. Gardner. Journ. Physiol., 1912, xliii, 401.
(47) Campbell, J. A. Journ. Physiol., 1923, lviii, Proc. vii-viii.
(48) Bayeau, R. C. R. d. l'Acad. d. Sci., 1921, clxxii, 1388; clxxiii, 937.
(49) Wright, A. W. Amer. Journ. Path., 1930, vi, 87.
(50) Wolbach, S. B. Bull. Johns Hopkins Hosp., 1919, xxx, 104.
(51) Campbell, J. A. Journ. Physiol., 1924, lix, 1.
(52) Tobiesen, F. Beit. z. klin. d. Tuberc., 1911, xix, 451; xxi, 109.
(53) Campbell, J. A. Journ. Physiol., 1927, lxii, 211.
(54) Burrell, L. S. T. and A. S. Macnalty. Med. Res. Council Spec. Rep. Series, 1922, lxvii.
(55) Abernethy, J. Surgical and physiological essays, London, 1793, 54.
(56) Leconte, Ch. and J. N. Demarquay. Arch. Gen. de Med., 1859, v, 111, 424, 545.
(57) Demarquay, J. N. Essai de Pneumatologie médicale, Paris, 1866. Trans. by Wallian, 1889, Davis, Philadelphia and London.
(58) Demarquay, J. N. and Ch. Leconte. Gazette medicale, 1863, 114.
(59) Sertoli. Med. Chem. Untersuchungen heraus v. Hoppe Seyler, 1866, iii, 350.
(60) Plumier, L. Travaux du Labatoire de Leon Fredericq, 1901, vi.
(61) Campbell, J. A. Journ. Physiol., 1926, lxi, 248.
(62) Bazett, H. C. and L. Sribyatta. Amer. Journ. Physiol., 1928, lxxxvi, 565.
(63) Campbell, J. A. Journ. Physiol., 1929, lxvii, Proc. vi-vii.
(64) Krogh, A. Skand. Arch. f. Physiol., 1904, xv, 328.
(65) Krogh, A. Skand. Arch. f. Physiol., 1913, xxix, 29; Pflüger's Arch., 1920, clxxix, 95, 113.
(66) Adler, G. Skand. Arch. f. Physiol., 1918, xxxv, 146.
(67) Gaarder, T. Biochem. Zeitschr., 1918, lxxxix, 48.
(68) Haggard, H. W. and Y. Henderson. Journ. Biol. Chem., 1919, xxxviii, 71.
(69) Campbell, J. A. Journ. Physiol., 1925, lx, 347.

(70) Wintrich, M. A. Handbuch der speziellen Pathologie und Therapie, 1854, v, Abt. i, 311.
(71) Hamman, L. and M. L. Sloan. Johns Hopkins Hosp. Bull., 1913, xxiv, 53.
(72) de Boismont, B. Recherches sur les Pneumatoses. These de Paris, 1825, cci, 21.
(73) Solon, M. Arch. gen. de Méd., 1835, ii, T9., 470.
(74) Ewald, C. A. Charité-Annalen (pro 1875) Berlin 1877, 167.
(75) Hoppe-Seyler, G. Deutsch. Arch. f. klin. Med., 1890, xlvi, 105.
(76) Webb, G. B., G. B. Gilbert, T. L. James and L. C. Havens. Arch. Int. Med., 1914, xiv, 883.
(77) Tachau, H. and R. Thilenius. Zeitschr. f. klin. Med., 1916, lxxxii, 199, 209, 223.
(78) Dautrebande, L. and P. Spehl. C. R. Soc. d. Biol., 1922, lxxxvi, 970.
(79) Grass, H. Beit. z. kl n. d. Tuberk., 1920, xlvi, 46.
(80) Grass, H. and H. H. Meiners. Beit. z. Klin. d. Tuberk., 1922, li, 134.
(81) Campbell, J. A. Journ. Physiol., 1923, lvii, 273.
(82) Hill, L. and J. A. Campbell. Brit. Med. Journ., 1923, i, 752.
(83) Planer, J. Zeitschr. d. K-K. Gesellsch. d. Aerzte zu Wien, 1859, xxx, 464.
(84) Schöffer, A. Sitzungsb. d. k. Akad. d. Wissensch. Math. Naturw. Cl., 1860, xli, 589.
(85) Morin, E. Journ. de Chim. et de Pharm., 1864, xlv, 396.
(86) Ewald, C. A. Arch. f. anat. physiol. und wissensch. Med., 1873, 1.
(87) Krogh, A. Respiratory exchange of animals and man, 1916, 77, Longmans, Green & Co.
(88) Buckmaster, G. A. and H. R. B. Hickman. Journ. Physiol., 1926, lxi, Proc. xvii–xvi ; 1928, lxv, Proc. xv–xvi.
(89) Campbell, J. A. Journ. Physiol., 1928, lxv, Proc. xviii–xx.
(90) Mainzer, F. Münch. klin. Wochenschr., 1928, xxvii, 1277.
(91) Berthelot. C. R. de l'Acad. Sci., 1900, cxxxi, 547.
(92) Heyse, E. Zeitschr. f. klin. Med., 1894, xxiv, 130.
(93) Senator, H. Internat. Beit. z. wissensch. Med., 1891, iii, 319.
(94) Rosenheim, T. Fortschrit. d. Med., 1887, v, 345.
(95) Dähnhardt, C. Arch. f. path. Anat. u. Physiol., 1866, 55.
(96) Hensen, V. Arch. f. path. Anat. u. Physiol., 1866, 68.
(97) Hammarsten, O. Ber. d.k. Sachs gesellsch. d. wissensch. Math. phys. Cl., xxiii, 1871, 617.
(98) Tschirew, S. Ber. d.k. sächsgesellsch. d. wissensch. Math.-phys. Cl., 1874, xxvi, 120.
(99) Buchner, H. Arb. a.d. physiol. Anstalt zu. Leipzig, 1876, xi, 108.
(100) Gaule, J. Arch. f. Anat. f. u. Physiol., 1878, 469.
(101) Ewald C. A. Arch. f. Anat. Physiol. u. wissensch. Med., 1876, 422.
(102) Pembrey, M. S. Schäfer's Text book of physiology, 1898, i, 783.
(103) Külz, R. Zeitschr. f. Biol., 1887, xxiii, 321.
(104) Barcroft, J. Biochem. Journ., 1906, i, 1.
(105) Setchenow. Zeitschr. f. rat. Med. v. Henle v. Pfeuffer, 1861, x, 285.
(106) Thörner, W. Maly's Jahresb., 1894, xxiv, 221.
(107) Külz, E. Zeitschr. f. Biol., 1895, xxxii, 180.
(108) Simpson, W. W. and J. J. R. Macleod. Journ. Physiol., 1927, lxiv, 255.

(109) KROGH, A. Journ. Physiol., 1919, lii, 457.
(110) HENDERSON, L. J. Blood: a study in general physiology. 1928, Yale Univ. Press.
(111) LINHARD, J. Pflüger's Arch., 1915, clxi, 233; with KROGH, Skand. Arch. f. Physiol., 1912, xxvii, 100.
(112) COHNHEIM, J. UND A. V. SCHULTHESS-RECHBER. Arch. f. Path. Anat., 1881, lxxxv, 503.
(113) GROSS, L. AND A. J. CLARK. Journ. Physiol., 1923, lvii, 457.
(114) GAARDER, T. Biochem. Zeitschr., 1918, lxxxix, 94.
(115) SCHIERBECK. Skand. Arch. f. Physiol., 1892, iii, 437.
(116) YLPPÖ, A. Münch. Med. Wochenschr., 1916, lxiii, 1650.
(117) EDKINS, N. Journ. Physiol., 1922, lvi, 421.
(118) DUNN, A. D. AND W. THOMPSON. Arch. Intern. Med., 1923, xxxi, 1.
(119) ERMAN. Gilbert's Ann. d. Phys. u. Chem., Leipzig, 1808, xxx, 113.
(120) LEYDIG. Arch. f. anat. Physiol. u. wissensch.-Med., 1853, 3.
(121) JOBERT. Ann. d. sc. nat. Paris, S. Zool., 1877, vi, T. 5, No. 8.
(122) POPOFF. Berlin klin. Wochensch., 1870, xxxvi i–xl, 453, 466, 477.
(123) KUHN, F. Zeitschr. f. klin. Med., 1892, 572.
(124) HOPPE-SEYLER, G. Deutsch. Arch. f. klin. Med., 1892, l, 82.
(125) KANTOR, J. L. Amer. Journ. Med. Sci., 1918, clv, 829.
(126) PLANER. Sitzungsb. d. k. Akad. d. Wissensch., Wien, 1860, xlii, 307.
(127) TAPPEINER, H. Zeitschr. f. Biol., 1883, xix, 228.
(128) TAPPEINER, H. Zeitschr. f. Biol., 1884, xx, 52.
(129) TAPPEINER, H. Zeitschr. f. physiol. Chem., 1882, vi, 432.
(130) HARDEN, A. Alcoholic fermentation. 1914, Longmans Green & Co.
(131) BOKAY, A. Zeitschr. f. Physiol. Chem., 1877, i, 157.
(132) HASEBRÖCK, K. Zeitschr. f. Physiol. Chem., 1888, xii, 148.
(133) STEPHENSON, M. Bacterial metabolism. 1930, Longmans, Green & Co.
(134) MACFAYDEN, A., M. NENCKI AND N. SIEBER. Journ. Anat. and Physiol., 1891, xxv, 390.
(135) MOORE, B. AND D. P. ROCKWOOD. Journ. Physiol., 1897, xxi, 373.
(136) MAGENDIE. Ann. de Chim. et Physiol., 1816, ii, 292.
(137) TAPPEINER, H. Arb. a. d. path. Inst. zu München, Stuttgart, 1886, i, 215.
(138) RUGE. Sitzungsb., d.k. Akad. d. Wissensch. Wien., 1861, xliv, 739.
(139) SCHMIDT, A. Deutsch. Arch. f. klin. Med., 1898, lxi, 545.
(140) FRIES, J. A. Amer. Journ. Physiol., 1906, xvi, 468.
(141) HOFMANN, K. B. Wien med. Wochenschr., June 15, 1872, 601.
(142) TAPPEINER, H. Ber. d. deutsch. Chem. Gesellsch., 1881, 2375.
(143) BUNGE, G. Text-book of physiological and pathological chemistry. Transl. by F. A. Starling, 1902, 476.
(144) BERGEON, L. C. R. Acad. d. Sci., 1887, civ, 1812.
(145) HANRIOT, M. AND CH. RICHET. C. R. Soc. d. Biol., 1887, viii, 307.
(146) ZUNTZ, N., F. LEHMANN AND O. HAGEMANN. Arch. f. Physiol., 1894, 351.
(147) FLINT, A. Med. News, Philadelphia, 1887, li, 670.
(148) BERNARD, C. Lecons sur les Effets des Substances toxiques et medicamenteuses. Paris, 1883, Lecture, 1859, 59.
(149) KROGH, A. Zeitsch. f. physiol. Chem., 1907, l, 289.
(150) OPPENHEIMER. Zeitschr. f. physiol. Chem., 1906, xlviii, 240.

(151) Campbell, J. A. Journ. Physiol., 1927, lxiii, 325.
(152) Campbell, J. A. Journ. Physiol., 1929, lxviii, 81.
(153) Campbell, J. A. Journ. Physiol., 1928, lxv, 255.
(154) Campbell, J. A. Lancet, 1926, i, 72.
(155) Schott, A. Proc. Roy. Soc. Med., 1928, xxi, 589.
(156) Campbell, J. A. and H. W. Dudley. Journ. Physiol., 1924, lviii, 348.
(157) Campbell, J. A. Journ. Physiol., 1929, lxviii, Proc. vii–viii.
(158) Campbell, J. A. and L. Hill. Journ. Physiol., 1924, lviii, Proc. xxv–xxvi.
(159) Campbell, J. A. Journ. Physiol., 1928, lxvi, Proc. i–ii.
(160) Campbell, J. A. Journ. Physiol., 1925, lix, 395.
(161) Campbell, J. A. Journ. Physiol., 1923, lvii, 386.
(162) Pantin, C. F. A. Proc. Roy. Soc. B, 1930, cv, 555.

LOCAL REACTIONS IN SENSITIZED ANIMALS

ARTHUS PHENOMENON, "HYPERERGIC INFLAMMATION"

MARTIN NORDMANN[1]

The Pathological Institute, University of Tübingen, Germany

In 1903 Arthus (1) published an experiment which is now known as the Arthus phenomenon. This experiment shows that one or several subcutaneous injections of serum change the local reactions of the skin to subsequent injections of the same kind of serum. The first injection produces a soft infiltration, which rapidly disappears, but when repeated again and again at certain intervals the skin reaction becomes more and more intense, the swelling no longer disappears and necrosis of the skin is the final result.

The methods of serology, morphology and pathological physiology have been applied in many investigations dealing with this reaction. In general smaller animals have served as the most suitable object for study; in addition certain observations in human beings have aided in this work. This review considers those papers which have contributed to the analysis of the mechanism of this phenomenon and it is the particular purpose of the last portion of this review to show how the understanding of this reaction has become of significance for the problems of general pathology.

It will be necessary first to describe somewhat in detail *the original experiment of Arthus.* He observed that one injection of horse serum under the skin of the rabbit produced a pale swelling at the site of the injection, and that the infiltration was absorbed in a few hours. The next injections at six days intervals had the same effect, but after the fourth injection the skin at the site of the swelling became pink and the swelling remained for two or three days. After the fifth injection the infiltration was hard and lasted during a period of five or six days. A white puslike center appeared in the swollen area after the sixth injection and the seventh injection caused necrosis of the skin, which healed only after a delay of several weeks. As far as the results are concerned it is immaterial whether the first injections are given in-

[1] I am greatly indebted to Dr. Leo Loeb for revising my paper.

travenously or intraperitoneally and whether fresh or preserved, active or inactivated horse serum is used. According to Arthus the reaction is specific, which means that the typical sequence of changes takes place only if the same kind of serum is used throughout the whole experiment.

Arthus could obtain necrosis of the skin of the abdomen as well as of the chest of the rabbit, but he did not succeed in obtaining this result with the same regularity when using the ear skin of the rabbit or injecting other kinds of animals. Arthus and Breton (2) studied also the various microscopical changes which took place under these conditions; but this phase of the problem will be discussed later.

This fundamental experiment was soon afterwards named the "Arthus phenomenon" by Nicolle (3) and since these first investigations many have repeated this experiment and have essentially obtained the same results.

Although this phenomenon was discovered by Arthus while he was studying anaphylactic reactions and although it bears close relations to the general problem of anaphylaxis we will not enter into a general discussion of those numerous reactions which occur when animals are repeatedly treated with the same antigen, but we shall strictly confine our discussion to those experiments which have been carried out more or less according to the fundamental experiment of Arthus. On the other hand, we shall consider not only the changes which take place at the site of the injection and in particular in the skin, but also the behavior of the blood, nervous system and other tissues under similar conditions. Then we shall have to discuss certain modifications which were made in the original experiment of Arthus: various proteins, vaccines or bacteria have been applied instead of serum and the number of injections varied, six or more injections being given in different experiments; but all the experiments had in common that several preliminary injections were made in order to sensitize the animals and that they were followed after a certain interval by a final injection which then called forth the characteristic result.

As mentioned above, Arthus found that his phenomenon could be elicited in the abdominal wall as well as in the skin of the chest of rabbits. Subsequent investigators attempted to extend *these experiments to other animals or to human beings.* It has been found that the phenomenon is not produced with equal readiness in all the animal species. Von Pirquet and Schick (4) observed necrosis of the skin in one patient among many others who had received several injections of serum. Lucas and Gray (5) described necrosis in the skin of children who had been treated

prophylactically with injections of antidiphtheric serum. An accidental observation in an adult woman has been reported by Hegler (6); in this case frequent injections of serum were followed by the formation of a cutaneous ulcer. However local edema is more often found under similar conditions than ulceration. Bouche and Hustin (7) injected small doses of serum into a great number of patients at various intervals. The sensitization remained for years when the injections of 1 ccm. in each case were repeated about twice a fortnight. Tonieti (8) gave daily injections of horse serum into the skin of patients and found local edema of an anaphylactic nature following the sixth injection, but did not observe necrosis. The same results were reported by Makai (9) in children. Gerlach (10) sensitized his own body with one injection of horse serum and obtained an intense infiltration one hour after the reinjection. This is not an exact reproduction of the Arthus phenomenon, which, as a whole, does not frequently occur in man.

As far as other animals are concerned the guinea pig occasionally shows the true Arthus phenomenon according to Nicolle (3) and Lewis (11). In general this animal reacts with definite edema which remains for days. In white rats the reaction does not at all reach the severity noted in rabbits. Some authors (Opie, 12) could not obtain even a trace of edema in these animals. We have to refer again to these results in guinea pigs and rats and other animals when reporting on the microscopical changes noted in the affected tissues. Observations in dogs are somewhat incomplete. Opie (12) has not observed any local reaction after sensitization of two animals either with serum or with egg white. Gerlach (10) on the other hand stated that he found certain changes, indicating a local reaction in dogs when he treated the animals with injections of small amount of antigen. A superficial necrosis of the skin in a goat following the seventh injection is mentioned by Opie (12). On the whole the rabbit is the most suitable animal for the study of the Arthus phenomenon and gives the most uniform reactions. At any rate the different reactions in different animals indicate that there is no direct ratio between the readiness of the phenomenon to occur and the readiness of an animal to become generally anaphylactic. As a matter of fact in some cases the Arthus phenomenon did occur in man or the reaction was at least similar to that in the rabbit. Therefore the phenomenon has become one of the most important experiments for the explanation of certain pathological reactions in man, which formerly could not be understood.

We shall now discuss somewhat in detail *the microscopical changes*

in the injected area of the skin. Twenty-four hours after the last injection Arthus and Breton (2) found edema with many leucocytes in the affected area and the connective tissue fibers had doubled in volume. After forty-eight hours, when gangrene was evident macroscopically, the infiltration had reached the surface, while the subcutaneous exudate showed extension and had diffusely infiltrated the tissue, the connective tissue fibers had become transformed into a homogeneous mass and there were hemorrhages in the deeper layers. The injured area was sharply outlined at that time. This condition remained stationary for about two days, when the skin sloughed off and the floor of the ulcer became covered with a layer of soft cheesy material which contained disintegrated leucocytes.

Much later Gerlach (10) published a very complete microscopical study of the Arthus phenomenon. He compared the end stage described by Arthus with the changes following preliminary injections. Even the first injection of serum into a normal animal elicits certain reactions and Gerlach noticed the appearance of leucocytes as early as the first few hours after the injection. After twenty-four hours he observed a slight reaction on the part of the connective tissue cells, also phagocytosis, and demonstrated the presence of small necrotic areas surrounded by leucocytes. However, it is not our intention to give here a detailed account of the reactions known as inflammatory because the anaphylactic reactions occuring under the condition of the Arthus phenomenon do not differ from the inflammatory reactions except in degree, their course being more rapid and intense; but there are no reactions which are in any way specific.

Gerlach (10) made another important observation. It had been stated by previous authors that the rabbit's ear failed to show the final necrosis characteristic for the phenomenon. Similarly the skin of the guinea pig and the white rat was believed to show almost no reaction. But when the areas of injection and reinjection were studied microscopically there was no difference observed in the reaction of the skin in the white rat and the rabbit's ear on the one hand, and in the abdomen of the rabbit on the other hand. Everywhere the affected areas showed connective tissue fibers which had become a homogeneous material; stasis, necrosis of the wall of the capillaries and other vessels, edema, leucocytosis and so on were noticeable. Thus the necrosis of the skin had lost much of its importance as a characteristic feature. We shall see below that as a result of these observations the original Arthus phenomenon was interpreted merely as an example of the so-called hyperergic inflammation.

In accordance with these conclusions are *the results obtained in organs other than the skin.* Thus Klinge (13) reinjected horse serum into joints of both normal and sensitized rabbits. He found that normal rabbits gave a very slight reaction, but that the sensitized animals showed a phlegmonous infiltration of the cavity and the capsule of the injected joints. Hepler and Simmonds (14) examined kidneys of rabbits which had been treated with horse serum or egg albumen. The control animals which received one single injection into the kidney, showed only hemorrhages, whereas in the sensitized rabbits a marked infiltration in these organs was noticeable. Opie (12) observed that rabbits sensitized by preliminary injections of horse serum and reinjected into the lung show necrotic areas which are surrounded by leucocytes. According to Friedberger (15) guinea pigs sensitized by one injection of horse serum develop pneumonic changes, when they are subsequently allowed to inhale a spray of horse serum. These changes consist in a proliferation of cells lining the alveolar walls and in exudation of eosinophilic leucocytes and of fibrin (Schlecht and Schwenker, 16). Shapiro and Ivy (17) using rabbits and dogs, reinjected sensitized animals into the mucosa of the stomach and obtained ulcers. We may also cite the experiments of Long and Seyfarth (18), who found that there was a more marked, although still mild infiltration with mononuclear cells in the testicles of sensitized guinea pigs, as compared with the condition in the normal animal.

A series of experiments carried out by Roessle (19) is of special interest. He observed the changes in the peritoneal cavity of reinjected guinea pigs directly through a window placed in the abdominal wall of the animal. Under these conditions sensitized animals showed a very definite inflammation of the peritoneum while in normal ones no noteworthy reaction appeared. This was the first experiment to prove functional variations in the course of the Arthus phenomenon in the living animal. Other experiments similar to that of Roessle we shall mention later; here we merely wish to state that the peritoneum as far as this phenomenon is concerned behaves not differently from any other organ.

Before we summarize the results of such morphological studies, we must mention certain observations of Möllendorff (20) which indicate, according to this author, that certain differences exist in the morphology of the connective tissue cells in sensitized and normal animals. He described in the former contraction of these cells, and vacuolization of their cytoplasm and—in the omentum majus of the guinea pig—a varia-

bility in the appearance of the superficial endothelial cells. However Finkeldey (21) emphasized that it is by no means possible to recognize sensitization from a microscopical section of any part of the connective tissue. Other histologists like Fischer-Wasels (22) and Maximow (23) have definitely denied the conclusions of v. Möllendorff. This discussion touches a very delicate problem, namely, that of the potency of fibrocytes. It is not yet certain that those changes and reversible formations of the fibrocytes which v Möllendorff continues to claim as specific for sensitization are really specific for this condition. However, this question cannot be discussed, before we have reported on other results concerning the mechanism of this phenomenon.

In the preceding part of our paper we have reviewed investigations which may be considered purely morphological and as depending solely upon the *use of histological methods*. We may summarize the results so far obtained as follows: The original Arthus phenomenon could be reproduced not only in the skin, but in many other organs. The changes occur also in animals other than the rabbit, and what is most important, also in man. The production of necrosis in the skin, to which so much attention was given by the earlier investigators, is limited to a small group of experiments, and the microscopical studies have shown that it is only an incident in a series of changes in the tissues, which do not differ from those occurring in the common process of inflammation but in degree, and furthermore we have seen that this process starts immediately after the first injection of the antigen. Important as these results are, it must be recognized that the methods of pure morphology could not carry us much further in the analysis of the mechanism of the phenomenon. Microscopical studies cannot give any explanation of the real causes on the changes which have taken place in the tissue; they show merely a final stage of what in some cases represents a long chain of events which usually are interpreted in a more or less imaginative way. The term "sensitization" and, as we will see below, also the term "hyperergic inflammation" represent conceptions which are based on macroscopical and microscopical pictures of the local process or on the success of the host in his effort to limit the reaction to a circumscribed area and to eliminate the foreign body, in a manner which serves best the restitution of the host organism to his normal state.

The application of other methods and in particular various modifications of the original experiment were necessary for further analysis and this extension of the work has contributed much to our knowledge of these problems. In the first place we have to refer to *the contributions*

made by serology. Arthus (1) had already concluded that the injected blood serum as such was not of great importance in determining the specific effects. Since 1 ccm. or less was sufficient to give a positive result in the later injections the effects could not possibly be attributed to an accumulation of the foreign protein within the body. Moreover, Nicolle (3) succeeded in producing the phenomenon in rabbits by means of passive sensitization, provided he injected as large an amount as 60 ccm. of serum from a sensitized rabbit into the peritoneal cavity of a normal animal. The demonstration of passive sensitization suggested that the phenomenon might be referable to the presence of a specific antibody, or according to Opie (12), to the presence of several antibodies in the serum. Now Opie found a fairly close parallelism between the changes in the precipitin content of the serum during the process of sensitization and the severity of the Arthus phenomenon. In the beginning of the experiment no precipitin was as yet noticeable in the serum, and likewise when the injections of the serum were discontinued at a later period, the precipitin disappeared again after two or three months. Between these two periods the precipitin reaction was positive, but unfortunately the parallelism between these two reactions is not perfect, because a definite Arthus phenomenon may be observed on reinjection even at times when no precipitin can be found in the serum.

Opie discusses the possibility that the precipitin is the antibody responsible for the Arthus phenomenon, and that the reaction takes place when the reinjected antigenic protein and the precipitin interact in the tissue. The interaction of both, antigen and precipitin, seems obvious, since the intravenous injection of precipitin is readily tolerated by the animal, but the simultaneous subcutaneous injection of foreign serum and the serum of an animal which had been previously sensitized against it causes an acute inflammation in the skin of a normal animal. The same occurs after the injection of precipitate, which developed when the two sera were mixed in vitro. It should then be expected that it is possible to saturate the antibody, which is supposed to be present in the tissue, by the antigen and to obliterate the phenomenon in this way. As a matter of fact the administration of a massive dose of a comparatively pure protein, e.g., crystalline egg albumen, caused the complete disappearance of precipitin formed in the blood as the result of previous injections of the same substance and gave rise to a definite desensitization of the animal concerned (Opie, 12). The same fact had been previously shown by Grineff (24) for egg white. However, when Opie

and others (Knox, Brown and Moss, 25), used such a complex substance as horse serum, they were not able to demonstrate a desensitization.

The precipitate formed by the combination of antigen and antibody in the tissues causes them and explains according to Opie the local anaphylaxis. However, in interpreting these experiments we have to distinguish between the facts which are actually established and the interpretation, which may be of a hypothetical nature. In this case there is lack of an exact correlation between the precipitin titres found in the blood and the severity of the inflammatory reaction in the skin, although within a certain range such a correlation exists. Opie himself explains the deviation by assuming that "the occurrence and the severity of the specific reaction might be modified by factors which have no relation to the antigen and the antibody concerned." It can indeed be shown that numerous factors of this kind do exist, and they cannot be discovered by the use of serological methods. By means of the precipitin reaction Opie has confirmed the fact already demonstrated by Rössle (19), which we shall discuss below, that antigenic proteins in sensitized animals are somehow fixed at the site of entry since they are not found in the blood stream. Opie admits the possibility that they are destroyed in the course and as a result of the inflammatory reaction which occurs at the site of the injection.

This review of the investigations carried out by means of serological methods proves that certain changes in the blood and tissue fluids occur in the Arthus phenomenon. They suggest the presence of a chemical substance throughout the body, the appearance of which precedes the morphological alterations of the tissue. These results without doubt have increased our knowledge of the mechanism of the Arthus phenomenon.

We shall now report on a number of experiments in which *certain modifications in the investigation of the Arthus phenomenon* have been successfully employed. Roessle (19) was the first to introduce such a modification which proved to be very useful. He wished to analyse the delay in the absorption of the antigen following later injections of this substance, and for this purpose he injected a visible antigen and examined the result in microscopical sections. He found erythrocytes of the chicken very suitable and when he injected them both into normal and sensitized guinea pigs, he noticed that the absorption in the sensitized animals took place with diminished rapidity, and that the cells wandering to the site of injection disintegrated at once. Among these cells of the host he observed a large number of eosinophilic leucocytes,

although the appearance of these cells did not prove to be a consistent reaction in sensitized animals.

These observations suggest a close relationship of these experiments with the work of Metschnikoff (26) and his followers, who still attribute the formation of antibodies circulating in the blood to phagocytic cells. By means of vital staining, as developed by Goldmann (27) and Aschoff (28), it could be shown that it was a certain group of cells within the body which usually acted as phagocytes, namely, cells belonging to the so-called reticulo-endothelial system. It was known that the intravascular injection of colloidal vital dyes and other colloids modifies the course of the general anaphylactic reactions and this fact was interpreted as due to a more or less intense depression of antibody formation by the histiocytes (phagocytes) after they had been blockaded by the dye. Roessle was the first to apply the conceptions of Metschnikoff to the Arthus phenomenon. The increasing intensity of the local reaction in the skin following later injections of the colloid substance, the typical sequence in the appearance of cells predominating at the point of inflammation and the decrease in the speed of absorption in later injections suggested to him that the tissue of the repeatedly injected animal had become more sensitive to the action of the substance used for injection than that of the normal animal. Therefore he distinguished what he designated as the normergic reaction from the *hyperergic reaction* or, to use the terms applied by Roessle, the normergic inflammation from the hyperergic inflammation. This point of view of Roessle has since become a very common explanation for various reactions of the organism to the antigen after the latter had been repeatedly injected. In this connection it must be understood that Metschnikoff and Aschoff as well as formerly Roessle himself confined the problem of sensitization to the activities of cells. There can be no doubt that the intense severity of the reactions after later injections suggests a kind of hyperenergy on the part of the body. However, as we indicated above, this interpretation is of a philosophical nature. The authors who had applied histological methods and had assumed that the processes observed under these conditions are due to the activities of cells visible at the site of the reaction, consequently arrived at the conclusion that these particular cells are more or less hyperergic. On the other hand, Opie criticised this assumption, because he found that antigen and antibody introduced simultaneously into the tissue produce the same inflammatory reaction in both normal and sensitized animals. He claims that the phenomenon is incorrectly designated as skin hypersensitiveness. According to Opie

the phenomenon rather depends upon the concentration of antigen and antibody which may be secondarily controlled by the supply of blood at the site of injection. The studies of Gerlach (10) which were undertaken at the suggestion of Roessle have shown that the hyperergic inflammation differs only in degree from the ordinary normal inflammation. Both the connective tissue and the vascular system differ as far as their functional state is concerned in sensitized and non-sensitized animals. Obviously Opie and Roessle and his associates agree that certain changes take place in the sensitized animals previous to reinjection. Opie, as stated above, has found the presence of precipitin in the serum, whereas Roessle and Gerlach, observing the different character of the reaction after sensitization, assume a change in the irritability of that tissue which in general is engaged in processes of inflammation. We will consider the evidence given in favor of the latter explanation when we report on the results obtained in the study of the Arthus phenomenon by means of methods of pathological physiology. In this place we shall merely state once more that Opie denies the change in local or general irritability of the tissue, however, without having actually determined this point in his experiments. The effects of the simultaneous introduction of antigen and antibody under the skin of the normal animal cannot be accepted as an exact reproduction of the Arthus phenomenon nor as evidence against the existence of a change in the reactivity of the tissue. We may assume that the granular material stained by eosin which Opie has seen in the subcutaneous tissue at the site of the injection of the antigen-antibody mixtures, represents the precipitate itself which had been produced in vitro and which he injected after it had previously been washed. On the other hand, he failed to demonstrate the presence of granular material, when he introduced the two sera containing antigen and antibody respectively at about the same time but separately, so that the precipitation had to take place in the tissue itself. In accordance with this interpretation Gerlach saw at no time in his experiments the slightest suggestion of a precipitate when he studied the microscopical sections. This negative result can be readily understood, if we consider the well known fact that there is a difference in the mechanism of precipitation in vivo and in vitro (Michaelis and Oppenheim, 29). If precipitation occurred in vitro the precipitate remained at the site of the injection and could easily be traced in sections; if the sera were injected simultaneously into the living animal no precipitate was noticeable. The occurrence of precipitation in the living organism remains hypothetical; and Opie himself explains the absence of any precipitate

in his sections by the assumption that the antibody coming from the blood and the antigen injected into the tissue might meet in part within the endothelium of the wall of the vessels, and therefore remain invisible. Now, it is evident that the Arthus phenomenon cannot occur without the interference of the tissue; furthermore there can be no doubt that the behavior of the tissue differs even in normal animals under different conditions of experimentation, as might be expected for instance if the same chemical substances are administered in a different manner either mixed or separate. It is very probable that the simultaneous application of the two sera, namely, normal horse serum and the serum of an animal sensitized against horse serum on the one hand and the injection according to the original method of Arthus on the other hand have different effects. However, we will see below that the changes which take place in the blood, as revealed by serological methods, are by no means the only result of sensitization.

Opie uses as a further argument in favor of his interpretation the fact that the simultaneous injection of the two sera into a normal animal produces "the same inflammatory reaction" as is known to occur in the Arthus phenomenon. However, as we have already noted, histological studies on the Arthus phenomenon have shown that it has no specific morphological feature and we must remember that inflammation as such can be produced under different experimental conditions; on the other hand, ordinary inflammatory phenomena do not show the gradually increasing severity of symptoms which is the characteristic feature of the Arthus phenomenon.

We shall now discuss some additional modifications of the Arthus phenomenon. The relation which has been found to exist between the reticulo-endothelial system and general anaphylaxis suggests similar relations of this cell system to local anaphylaxis. In accordance with this conception the extirpation of the spleen is supposed to inhibit the anaphylactic shock. Accordingly Klinge (30) removed the spleen in dogs and rabbits, but observed that the sensitization of these animals could be obtained in the same way as in normal dogs or rabbits. He also tried to control the Arthus phenomenon by blockading the reticulo-endothelial cells by means of vital stains. For this purpose he injected at various times into the sensitized animal trypanblue subcutaneously at the site where the final reinjection of the antigen was to take place. As long as the dye was infiltrating and diffusely staining the pericellular tissue the reinjection of the antigen did not cause any local effect. This refractory condition continued until the first granules

and vacuoles could be distinguished within the protoplasm of the cells at the point of reinjection. Later on however when still more dye appeared in the cells in granular form, the effect of the reinjection of antigen no longer differed from what happened in the original experiment of Arthus or in the experiments of Gerlach respectively. When Klinge injected trypanblue intravenously, even a massive dose, such as is necessary for the blockade of the reticulo-endothelial system, could not change the course which the Arthus phenomenon took in the skin, provided the injection of the dye had been carried out a few days before the reinjection of the antigen. However, when trypanblue was injected within the last 48 hours preceding the reinjection of the antigen, the local reaction of the skin was lacking. Likewise when the dye was introduced within the last 48 hours before any injections of antigen, an efficient sensitization did not take place.

Klinge concluded therefrom that the vital staining as applied by him is not an indifferent procedure, but that trypanblue temporarily paralyzes the activity of the mesenchyme either locally or in the whole organism. In this connection we must add that in the meantime the theory of Aschoff as to the significance of the reticulo-endothelial system had been further developed by Siegmund (31). In the opinion of this author the site of the antibody formation and of parenteral metabolism is constituted by the entire mesenchyme of the body. Hence paralysis of the "active mesenchyme" might prevent any anaphylactic reaction. As a result of his own studies Klinge believes that the mesenchyme ceases to form antibodies or to react to the antigen as long as it is confronted by the dye present in the interstitial tissue. When the dye is taken up by the cells, the essential process leading to the storing of the foreign material may be finished and the mesenchyme may again be able to function and therefore produce anaphylactic reactions.

Klinge has thus again brought back the problem of local anaphylaxis to the action of cells, although he apparently interprets the mesenchyme as a connected whole in accordance with the view of Hueck (32). This hypothetical conception frustrates an analysis concerning the significance of the connective tissue in general and of the different types of cells in particular which might be engaged in the process, as well as of the rôle which the vascular and nervous system might play in it.

When *we summarize* all the papers which have been referred to in the last part of this review, we may state that *the studies of a purely morphological and serological character* have been greatly aided by the introduction of visible substances. Thus it has been possible to follow

microscopically the way of the absorption of the antigen within the body and to observe that the absorption was delayed in sensitized animals. The seat of the parenteral metabolism could be demonstrated to a certain extent by vital stains, and it could be blockaded for a very short period by trypanblue. These experiments lead therefore to the conclusion that in the tissue, specialized cells such as phagocytes or the connective tissue as a whole, might be hyperergic in the case of sensitization.

The results of the morphological and serological studies reported so far can be summed up as follows: the Arthus phenomenon has a widespread significance, its characteristic feature being the gradually increasing severity of the local reaction at the site of the injection of the antigen. Since it has no specific morphological characteristics, it seems but a certain type of inflammatory reaction, the difference from the usual inflammatory reaction consisting essentially, although not exclusively, in its being associated with the presence of a precipitin titer in the blood. As far as purely morphological studies can reveal the course of events, the rapidity in the succession of the individual phases of the inflammatory process is increased in sensitized animals. On the other hand, the absorption of the antigen is delayed at the site of the injection of the antigen in sensitized animals. Whether or not it is justifiable to apply the term hyperergic reaction, to the processes described, there can be no doubt that there is a change in the reaction to the later injections. From the studies mentioned above, we may conclude that it is the chemical composition of the blood and the metabolism of the cells of the connective tissue which are most probably altered by the sensitization. However, since no causal connection between these two effects could be found the interpretation of the mechanism of the phenomenon remained a matter of opinion of the various authors in this field of investigation.

We now turn to *results obtained by* still another method which has been applied to the study of our problem, namely, *pathological physiology*. There are many suggestions in the literature that the phenomenon might be a result of an interference with the blood circulation. These conclusions are mostly based upon the general behavior of the sensitized animal. Morphological observations and the results of serological experiments have been used in a more or less hypothetical manner for the interpretation of the rôle which the circulation may play in the Arthus phenomenon. An early paper dealing with this aspect of the problem was published by Fröhlich (33) who worked in Roessle's labora-

tory. Fröhlich examined the mesentery of the frog according to the method of Cohnheim. He found that the circulation of a sensitized frog immediately stops when the mesentery is brought in contact with specific serum either in a liquid or a dry state. The affected area then becomes anemic and is surrounded by a zone in which an abundant emigration of leucocytes from the blood vessels takes place. Very soon the picture of a general inflammation was fully developed. Again, the severity of this circulatory reaction which was so intense in the living organism, was believed to be the result of an hyperergic reaction.

Some interesting experiments bearing on this aspect of the problem were published by Auer (34). This investigator applied pure xylol to the ear of a sensitized rabbit for a period of from 15 to 30 seconds. He noticed a very severe edema and necrosis at the tip of the ear which developed within seven to ten days. In these experiments the application of xylol to the ear of the rabbit took place three hours after a massive dose of foreign serum had been injected into the peritoneal cavity of the animal. As xylol causes a very marked hyperemia of the ear the author believes that under the condition of his experiments the sensitized organism injects itself with antigen at the place treated with xylol. However, we shall see that these experiments allow quite a different explanation; what they really show is that a non-specific product elicits a more intense reaction in a sensitized than in a normal animal. Or to express it differently, in a sensitized animal a local anaphylactic reaction may take place even when a substance other than the one used for sensitization is injected.

Tannenberg (35) repeated this experiment using cantharidin instead of xylol and found the reaction usually called forth by cantharidin to be decreased in sensitized as compared with normal animals. The opposite result was thus obtained with cantharidin to that obtained with xylol. We would interpret all these experiments as indicating that sensitized animals have an altered readiness to react to various substances but that the direction in which the change occurs may vary according to different conditions of experimentation. We arrive therefore at the conclusion that the Arthus phenomenon does not signify a specific antigen reaction. Moreover, these experiments do no longer admit of the interpretation of this process as an hyperergic inflammation, since the reaction observed after cantharidin was obviously diminished rather than intensified in the sensitized animals. There are numerous other experiments and observations which demonstrate that under certain conditions the organism may react either with increased or with a de-

creased readiness to a certain substance. The best known example of this kind is the immunity against infectious diseases which follows the first infection, or the desensitization by means of pure antigen as demonstrated by Opie (12). Another example well known in general pathology is the ability to consume morphine in gradually increasing doses with the long continued use of this alkaloid. As repeatedly stated above, the severity of the local reaction of the Arthus phenomenon as manifested in necrosis does not necessarily prove that the phenomenon is hyperergic in character. In the discussion of his experiment with cantharidin Tannenberg (35) has suggested that the tonus of the arteries might be increased as the result of the repeated injections of the antigen, because he noticed that the site of the application of cantharidin was very pale, and that the local hyperemia usually associated with the inflammatory reaction to cantharidin in normal animals, was inhibited by the hypertonic state of the arteries in sensitized animals. However, so far we have to deal with theoretical discussions only, since a direct investigation of the behavior of the arteries in the course of the Arthus phenomenon had not yet been undertaken at that time.

Our own recent experiments which were carried out in coöperation with Dietrich (37) may throw some light on this aspect of the problem. We sensitized rabbits by means of intravenous injections of vaccines of bacterium coli. After an interval of seven to ten days at which time the optimal period for the local anaphylactic reaction has been reached, adrenalin was applied to the mesentery, because the peritoneum has been shown by Fröhlich (33) to be a suitable tissue for the demonstration of the phenomenon in question. However, this author used frogs in his experiments the circulation of which is obviously different from that of the rabbit. In our work the circulation was observed directly under the microscope according to the method of Kühne and Lee. We found that the constrictor reaction which adrenalin normally elicits was shortened in sensitized animals and, furthermore, that there was a difference in the reaction of the arteries on the one hand, and the terminal vessels (small arteries, capillaries, small veins) on the other hand. In sensitized animals the arteries remained in the contracted state only about one-half of the usual time and this phase was followed by a second phase in which rhythmical dilatation and contraction took place. It was different with the terminal vessels which in our experiments soon returned to their original size. We may thus consider as definite the fact that in sensitized animals the reaction to adrenalin is shortened in the circulation of the peritoneum which represents a place

at some distance from the site of the previous injection of the antigen; this proves that the effect on the circulation extends through the whole organism. In accordance with this result, we found that the blood pressure of sensitized animals does not respond with the same readiness to the intravenous injection of adrenalin as the blood pressure of a normal animal. This effect of sensitization seems to us the more important, since Tonieti (8) has obtained the same result in patients who had been treated with at least six daily injections of serum. They showed a definitely retarded reaction of the blood pressure after subcutaneous introduction of adrenalin. Both Tonieti and we have therefore concluded that continuous treatment with antigen diminishes the irritability of the whole nervous system, because it is the nervous system which regulates the blood circulation not only in the mesentery but all over the body. We have drawn this conclusion notwithstanding the fact that Klinge (30) has denied any participation of the nervous system in local anaphylaxis, because he could obtain the Arthus phenomenon in the ears of the rabbits after cutting both the sympathetic and the motor nerves. He failed however to eliminate the local nerve supply of the vessels which reacts independently of the central nervous system. This question has been discussed by us elsewhere and does not need to be considered any further in this connection. The nervous system of the circulation plays an important rôle too in the next part of our experiments and meanwhile we may keep in mind the fact that sensitization alters the irritability of the nervous system.

After this condition of the sensitized animals was established, we reproduced also the effect of the final reinjection of the antigen characteristic of the Arthus phenomenon by infecting the peritoneal cavity of rabbits with bacterium coli. Within a few minutes the true picture of inflammation developed under the eyes of the observers in both normal and sensitized animals. A reaction of this kind is associated with the rapid disappearance of the adrenalin effect which otherwise takes place in the rabbit, and thus within half an hour the vessels in the area of experimentation will no longer contract, except very large arteries which show a slight contraction of short duration. However, when the animal under observation has been sensitized previously, the arteries even in the inflammatory district do react, although their contractility as a result of sensitization is of course diminished as compared with normal conditions; but the subsequent infection obviously does not cause an additional diminution in the irritability of the arteries. Unlike the arteries, the terminal vessels lose their contractibility under

the influence of sensitization as they do under normal conditions of inflammation. If the infection of the peritoneum was accomplished by means of a smaller dose of bacterium coli and if the effect of the bacterial infection was at once examined microscopically, peritonitis was found to be the result in both normal and sensitized rabbits. But there was a definite difference in the later development of the peritonitis. While the normal animals survived for a period of only about seven hours, the sensitized rabbits lived as long as for twenty-one hours afterwards, therefore three times longer than the normal ones. At the expiration of a six hour period following the intraperitoneal injection the blood pressure of the originally normal rabbits was lower than it would have been under ordinary conditions and it did not rise appreciably when adrenalin was injected into the vein of the ear; on the other hand, the blood pressure of the sensitized animals remained normal up to twenty-one hours following the injection of the bacteria into the peritoneum. Furthermore, the reaction to intravascular injection of adrenalin was less pronounced in the sensitized animals than in normal rabbits under ordinary conditions, but it did not differ from the reaction as it takes place in a sensitized animal without a peritonitis. This is in accordance with expectation because the blood pressure is maintained by the arteries only and is independent of the condition of the terminal circulation.

Before discussing our results we must recall some well established facts of general pathology. Necrosis is due to a more or less complete obliteration of the lumina of the blood vessels which supply a certain area with blood. This obliteration usually occurs when stasis has developed in the terminal vessels. The arteries leading directly into this area ("vorgeschaltete Arterien") are more or less contracted under these conditions. Whether or not this contraction is a primary or a secondary process, is still a debatable question at the present time (cfr. the conclusion of Ricker (38) on the one hand and the conclusion of Tannenberg (39) on the other hand). However, the fact that such a contraction actually does take place is generally recognized, and this process is supposed to inhibit the circulation in the terminal vessels.

A similar condition is found in the circulation under the circumstances which commonly are designated as inflammatory. Here also the arteries are contracted and this vascular contraction causes a decrease in the rate of the blood current in the terminal vessels which are extremely dilated at that time. The more the arteries leading to the center of irritation resist the tendency to dilate, the more severe and prolonged is the inflammatory process in the terminal vessels. Whenever

the contraction of the arteries is complete or almost complete, the circulation in the terminal vessels stops entirely, stasis occurs and necrosis of the tissue results.

Now, returning to our own experiments we have noticed that the arteries resist more and more the tendency to dilatation during and following the reinjection of the antigen. It is then to be expected that the process of inflammation in the injected area is at first prolonged and finally results in necrosis, a sequence of events which corresponds exactly to what has been observed in the Arthus phenomenon. We conclude therefore that the cause of the final necrosis following the later injections in the Arthus experiment is due to the ability of the arteries to become accustomed to the serum and that in consequence of this adaptation they are less and less altered by additional injections of the antigen. Furthermore our own results and those of Tonieti show that the irritability of the nervous system, and in particular that of the nervous system of the circulation, is changed by the process of sensitization and we therefore do not hesitate to attribute the different behavior of the arteries to this changed attitude of the nervous system in normal and in sensitized animals respectively.

In interpreting the Arthus phenomenon in this way, we do not wish to maintain that there is a direct connection between the injection of the antigen and the changes which take place in the nervous system of the animal. On the contrary we consider it as probable that a very complex mechanism is responsible for this very definite alteration of the nervous system.

We will now *recall* once more *the principal results of the various investigations cited* above: the characteristic feature of the Arthus phenomenon consists in the gradually increasing severity of the local reaction to the antigen which has been injected repeatedly at certain intervals. Any part of the body which is suitable for injection may be affected that way. The changes can occur in many animals including man and in different organs the alterations begin with the very first injection. The reaction at the site of injections is inflammatory in character and does not differ in the later injections from that observed in the earlier injections except in degree.

The changes which could be established *as preceding the reinjections* and as therefore *due to the process of sensitization* are as follows: 1. An increased precipitin titer is fairly regularly associated with an efficient sensitization. 2. The irritability of the nervous system as a whole is diminished as the sensitization process progresses. 3. Cells of the con-

nective tissue show some alterations among which may be noted a not infrequent contraction of fibrocytes and vacuoles within their protoplasm.

We must admit that these results are insignificant when we consider the large amount of work which has been done to clear up the mechanism of the processes underlying the Arthus and allied phenomena. Moreover the results are far from being securely established. Thus the precipitin titer does not invariably accompany the process of sensitization, the changes in the cells of the connective tissue are not uniformly recognized as specific for the sensitization, the alteration in the irritability of the nervous system needs to be confirmed by the work of a larger number of investigators than has been done so far, especially in view of the fact mentioned by us that some authors have denied the rôle of the nerves, although on insufficient grounds. While under those conditions, we do not believe we are able to give a final interpretation of the mechanism of the sensitization process, we think it possible to *discuss in a provisional manner certain interpretations of the Arthus phenomenon which have been proposed by earlier investigators.* As stated above, it seems impossible for us to accept the view of Opie, who looked upon the Arthus phenomenon as merely a question of the concentration of antigen and antibody at the site of injection. That view above all denies an active participation of the body in the process; yet it is a very common experience of general pathology that any effect within the organism is not only due to the greater or less concentration of the stimulating factor, but in addition depends upon the general condition of the affected organism. We realize that Opie has expressed the opinion mentioned in such a definite way only because he wished to argue against the interpretation of Roessle who designated the Arthus phenomenon as a manifestation of an hyperergic condition. We freely admit that we quite agree with Opie when he declines to accept the explanation of Roessle. As stated above, we recognize this explanation as teleological, in as much as it assumes that necrosis is the best method for the organism to eliminate the foreign protein and that this usefulness explains the occurrence of this reaction. As regards the details of the mechanism underlying this process Roessle's explanation is erroneous, for, as we have shown, the nervous system is certainly not hyperergic in sensitized animals, since the reaction to adrenalin indicates a diminution rather than an increase in the irritability of the nerves supplying the vessels. The behavior of the arteries during the reaction following the final reinjection may be interpreted either as "hyperergic" or with

equal justification as "hypergic." Which of these two interpretations we choose, is to some extent arbitrary: the arteries may be considered as "hyperergic" in so far as they resist the tendency to become paralyzed by the antigen as they would have been without sensitization; on the contrary, they may be considered as "hypergic" in so far as they fail to react to the reinjected antigen. Whichever of these two definitions we should adopt, it would be a teleological interpretation. In our own paper we have generally used the expression "vorbehandelte Tiere" which means previously treated animals which seems to us to represent a more indifferent term than that of "sensitized animals." Finally we have to add a few words about the view of Klinge and others, who claim the "activation" of the mesenchyme to be the essential result of the sensitization. We cannot accept this interpretation as a satisfactory explanation. In this connection we must recall the fact that trypan blue has a very marked effect on the nervous system as well as on the blood circulation, as demonstrated by Klink (40) in Ricker's laboratory. The morphological changes which have been observed in the interstitial tissue and in the cells after injection of this substance never occur without a preceding interference with the local nervous system and the blood circulation, as pointed out by Ricker (38) and his collaborators.

In conclusion we wish to refer to some experiments of Dietrich (41) which in certain respects are related to the Arthus phenomenon, although they differ from the latter, in so far as the ultimate test injection was given intravenously and also inasmuch as the reinjected material was not exactly the same as that used for sensitization. Dietrich sensitized rabbits with vaccines or proteins (caseosan) and subsequently injected bacteria into their circulation. In a considerable percentage of these animals acute endocarditis followed the injection of bacteria into the ear vein. When at the same time the vena jugularis at the site of the injected ear was partly obliterated by artificial dislocation thrombosis of this vessel developed. Dietrich believes that under the conditions obtaining in his experiments the secretory function of the endothelium, including that of the heart valves, is altered. Moreover he believes that in addition the local disturbance of the circulation is an important factor. The mechanism leading to the effect observed by Dietrich cannot be discussed here, as it does not strictly belong to our theme, nevertheless these experiments illustrate still further the widespread significance of anaphylaxis in general and of local anaphylactic reactions in particular. They show that the possibility of a sen-

sitization playing a part in pathological processes must be considered also in such cases in which complicating factors in the blood or in the tissues may obscure the essential anaphylactic character of the reaction observed.

In human pathology the further analysis of the Arthus phenomenon and its implications has thus led to the recognition of the anaphylactic character of various pathological phenomena or diseases; among these we may especially mention the development of urticaria following repeated injections of a foreign serum. This observation led to the suggestion that the various types of exanthemata, which are characteristic of infectious diseases, may also be anaphylactic in nature. Moreover, we may consider as certain that the "rheumatic" arthritis, acute or chronic, represents a local anaphylaxis of the joints. The same applies to endocarditis and thrombosis of veins which are so generally associated with infectious diseases.

The Arthus phenomenon, in association with serological investigations as well as with morphological observations concerning the parenteral metabolism and especially in association with physiological investigations concerning the circulation and the nervous system, has grown to be a very important factor in the explanation of the variability of the reactions of the body which follow the same type of stimulation under different conditions. Much work still needs to be done before the problem of its mechanism can be considered solved. In this review, which is not intended as a complete report on all the papers dealing with this subject, our purpose has been primarily to show that the mechanism of the Arthus phenomenon, as well as that of all other biological phenomena, should be explained not from the point of view of either serology, morphology or physiology separately, but from that of all these sciences combined.

BIBLIOGRAPHY

(1) Arthus. 1903. Compt. rend. des scéances de la soc. de biol., lv, 817.
(2) Arthus et Breton. 1903. Compt. rend., lv, 1479.
(3) Nicolle. 1907. Ann. de l'inst. Pasteur, xxi, 128.
(4) v. Pirquet und Schick. 1905. Die Serumkrankheit. Wien.
(5) Lucas and Gay. 1909. Journ. Med. Res., no. 20.
(6) Hegler. 1923. Klin. Wochenschr., Nr. 15, 698.
(7) Bouche and Hustin. 1921. Presse med., Nr. 29.
(8) Tonietti. 1925. Zeitschr. f. d. ges. exp. Med., Nr. 45.
(9) Makai. 1922. Deutsch. Med. Wochenschr., 257.
(10) Gerlach. 1923. Virchow's Arch., ccxlvii, 294.
(11) Lewis. 1908. Journ. Exp. Med., no. 10.

(12) Opie. 1923. Journ. Immunol., viii, 19.
1924. Journ. Immunol., ix, 255, 231, 259.
1924. Journ. Exp. Med., xxxix, 659.
(13) Klinge. 1929. Ziegl. Beitr. z. Path. Anat., lxxxiii, 185.
(14) Hepler und Simonds. 1929. Amer. Journ. Pathol., v, 473.
(15) Friedberger. 1911. Deutsch. Med. Wochenschr., 481.
(16) Schlecht und Schwenker. 1912. Deutsch. Arch. f. Klin. Med., Nr. 58.
(17) Schapiro and Joy. 1928. Arch. Int. Med., no. 37.
(18) Rössle. 1914. Verh. d. Deutsch. Path. Gesellsch. 17, Tag.
(19) Long and Seyfarth. 1924. Amer. Rev. Tuberc., no. 9.
(20) Möllendorff. 1928. Zeitschr. f. Zellforschung u. mikr. Anat., vi, H. 1–2.
(21) Finkeldey. 1930. Krankheitsforschung B. viii, H. 4, 314.
(22) Fischer-Wasels. 1930. Verh. d. Deutsch. Path. Gesellsch. 25 Tag.
(23) Maximow. 1929. Ziegl. Beitr., lxxxii, 1.
(24) Grineff. 1912. Compt. rend. des. sci. de la soc. de biol., lxxii, 1.
(25) Knox, Brown and Moss. 1910. Journ. Exp. Med., no. 12.
(26) Metschnikoff. 1901. L'immunité dans les maladies infectieuses. Paris, Masson.
(27) Goldmann. 1909. Beitr. z. Klin. Chir., lxiv.
(28) Aschoff. 1924. Ergebn. d. inn. Med. u. Kinderh., xxvi.
(29) Michaelis und Oppenheim. 1902. Arch. f. Anat. u. Phys. Abt. Supl.
(30) Klinge. 1927. Klin. Wochenschr., 273.
1926. Krankheitsf., iii, 174.
1928. Krankheitsf., v, 458.
(31) Siegmund. 1927. Beihefte d. Med. Klin., H. 1.
(32) Hueck. 1920. Ziegl. Beitr., lxvi, 330.
(33) Fröhlich. 1914. Zeitschr. f. Immunitätsforsch., nr. 20.
(34) Auer. 1920. Journ. Exp. Med., no. 32.
(35) Tannenberg. 1926. Verh. d. Deutsch. Path. Gesellsch. 21 Tag, 144.
(36) Coca. 1919. Journ. Immunol., no. 4, 219.
(37) Dietrich-Nordmann. 1928. Krankheitsforsch., vi, 217.
1929. Krankheitsforsch., vii, 321.
(38) Ricker. 1924. Relationspathologie (Springer).
(39) Tannenberg. 1926. Franf. Zeitschr., xxxiii, 91.
(40) Klink. 1929. Zeitschr. f. d. ges. Exp. Med., Nr. 67, 219.
(41) Dietrich. 1926. Zeitschr. f. d. ges. Exp. Med., Nr. 85.
1930. Virchow's Arch., cclxxiv, 425.

FACTORS IN THE DEVELOPMENT OF SEX AND SECONDARY SEXUAL CHARACTERISTICS

OSCAR RIDDLE

Carnegie Institution of Washington, Station for Experimental Evolution, Cold Spring Harbor, N. Y.

It seems permissible to discuss this subject as it is stated above despite the salient fact that it is becoming logically impossible to disentangle "secondary" sex characters from anything else that pertains to sexuality. Practically, however, the term "secondary sex character" remains useful, and even a short review which attempts to place the various aspects of sexuality into their modern frame is perhaps of some value. Ignorance is still plentifully mixed with much precise knowledge, and any attempt to obtain a connected view of the widespread and intensely biological thing called sex is akin to adventure. Entangled everywhere are the cogent—yet apparently contradictory—principles of chromosomal sex determination on the one hand and of sex-reversibility on the other. These principles are often exhibited in such odd bed-fellows as: fixity and plasticity; hermaphroditism; gonad of one sex and other physical or psychical characteristics of the other sex; hormonal control, control by such special physical agents as temperature or light, perhaps even cases of no clear control whatsoever—either asexuality or a riot of sexuality. Since, however, the apparent contradictions now seem to begin to dissolve, and also to reveal an unusual variety and depth of present information concerning a certain aspect of development, the hazards of the task may be temporarily forgotten in an effort to evaluate and review this aspect of developmental physiology.

It is certain that two slightly but peculiarly different combinations of genes or genetic factors (carried in the chromosomes) are formed in fertilized germ cells, and that *in some way* this difference in genes (which is continued into practically every cell of the organism) normally decides that conjugating individuals (males) shall develop from one type of combination and reproducing individuals (females) from the other. Ultimately these same genetic factors *in some way* determine also that the "accessory" and the "secondary" sex characters of the developing individual shall be in conformity with the "primary" sex characters (testis or ovary). During the last two decades, however, it has become

equally certain that genetic combinations which would normally develop into a female can be forced, *in some way* or ways and at the will of the experimenter, to complete their development as males, and *vice versa*. That is, the reversibility of sex is a fact; indeed, it has been established as a principle of sexuality. In general it can be said that whenever the "primary" sex characters (gonads) are reversed the "accessory" and "secondary" characters are reversed also. For the reason that the primary sex character usually develops or differentiates *earlier* than do the so-called secondary ones it happens that reversals occur more often and more readily in the secondary than in the primary characters. At once therefore we face the hard-won fact that genetic (chromosomal) difference normally decides prospective sexuality, primary and secondary; but that this chromosomal difference is not the real or ultimate cause of sex difference—since usable agencies may secure either sex from the same chromosomal complex. Until this is recognized, and unless a search is made for that determiner which is common to the genes and to the effective agencies for sex-reversal, any discussion of the present subject is quite superficial and perhaps futile.

THE RELATION OF CHROMOSOMES TO SEX. In order to provide one part of the background against which the origin of everything concerned in sexuality must be traced, it is necessary to supply a brief outline of the method by which the germ cells are endowed with two slightly but significantly different chromosomal arrangements. These facts we owe to McClung (1902) and to a host of others. The actual situation varies considerably among different animal species, but that found in the human may well serve as a basis of the present description. In both sexes the sperms and egg cells have 24 pairs of chromosomes. Of these, 23 pairs are known as autosomes; the other pair is rather specially concerned in the determination of sex and these are therefore called sex chromosomes or X-chromosomes. The two members of the pair are exactly alike in the female (XX). In the male, however, the two sex chromosomes differ from each other; one is precisely like the X-chromosomes of the female, the other—called the Y-chromosome—is much smaller. Hence with respect to this pair of chromosomes the female cells are XX, the male cells XY. (Winiwarter and Oguma (1930) find no Y, i.e., only 23 chromosomes, in sperm of man.) The female cells which form eggs do so by a division of their 24 pairs into 24 singles, and thus every egg is provided with one X-chromosome. When male cells similarly divide to form sperms only half of the resulting sperms will possess an X-chromosome while the other half will bear the smaller

Y-chromosome. It is clear that when an X-bearing sperm unites with any egg whatever (all X-bearing) it will restore the female (XX) type of cell; and the evidence obtained from the breeding of many animal species clearly shows that females normally result from such cell unions. Clearly also when a Y-bearing sperm unites with any egg of its species (all X-bearing) it will restore the male (XY) type of cell and develop as a male.

The production of two kinds of sperm in the manner just described has been found to apply to mammals, some fishes, most insects, to sea urchins and some worms. But in birds, other fishes, moths and butterflies it is the male that contains the two similar chromosomes (here designated ZZ instead of XX) and the female has the two which differ ZW instead of XY). In these species therefore there are two kinds of eggs and only one kind of sperm. Within any of these species the above described mechanisms provide the genetic or chromosomal or genic basis for two sexual types, and these mechanisms and differences are of primary importance to an understanding of the origin of primary, accessory and secondary sex characters. The chromosomal differences which exist in each type of fertilized eggs (zygotes) are extended through cell division to every tissue and cell of the body.

It should next be noted that the Y-chromosome, though sometimes of large size, is believed to carry few or no genes. Also that it is the presence of this "empty" chromosome as the mate of an X which provides the basis for the appearance of *sex-linked* characters—such as color blindness, Leber's disease and hemophilia in man (recent study and review by Davenport, 1930). These latter usually are recessive traits, the genes for which are carried in the X-chromosomes, and the traits will develop only if no normal gene is present in the other sex chromosome of this chromosomal pair. Sperm cells that carry an "affected" X-chromosome become progenitors of daughters half of whose eggs, in turn, carry the affected chromosome. If such eggs produce males, these males will be affected; if females, their defective X-chromosome will be balanced by a normal X-chromosome derived from the sperm and these will be normal. Finally, since genes for characters apparently unrelated to sexuality can be carried in the sex chromosomes, it would seem that these so-called *sex-linked* characters fall quite outside the present review. This, however, is perhaps not entirely true as will be noted below.

From studies on the non-disjunction of chromosomes in *Drosophila* in 1916 Bridges supplied experimental proofs of the different and specific

rôles of the X- and Y-chromosomes in sex determination, and demonstrated that an X derived from a male is identical with an X from a female in its influence on sex determination. In 1922 and 1925 he could show further that the presence of two X-chromosomes do not necessarily lead to the production of a female as was formerly widely believed. Flies were found which were neither male nor female but sex intermediates—being a mixture of male and female characters. Both cytologically and genetically Bridges proved that these individuals possess two X-chromosomes and *three* sets of autosomes. It was thus made probable that the autosomes also influence the development of sex, and that *genes* rather than whole chromosomes are really involved. Indeed in *Drosophila* it was found that "the fourth chromosome has a *net* female tendency, similar to that of the X and different from that of the other (two) autosomes." Again, the X- and Y-chromosomes—according to the present expression of most geneticists—seem to exert their influence on sex determination partly or wholly through the circumstance that they carry unequal numbers of sex-linked genes, and fertilizations by X and Y therefore establish differences in the *proportion* of sex-linked to non-sex-linked genes. This conception of "genic balance" is rather widely accepted by geneticists as the method by which the genes influence the development of maleness and femaleness. This view was simultaneously greatly strengthened by Blakeslee's (1922) studies on chromosomal reduplication in *Datura*. In these plants the somatic cells normally have 12 pairs of chromosomes. Normal plants also develop when either 2, 3 or 4 full sets (24, 36 or 48 chromosomes) are present; but the presence of even a single chromosome in addition to the groups of 12, 24, etc., was found greatly to "unbalance" the development of many characters.

It should be noted that in the American work with *Drosophila* (partly cited above) on the relation of chromosomes to sex, and on chromosomal conditions in intersexuality, there was unreadiness to grant a basis for quantitative sexuality prior to 1922, and that such a quantitative chromosomal basis had been proposed much earlier by Goldschmidt (1912, 1917) and Harrison and Doncaster (1914) in their work on *Lymantria*. For the part of the preceding account which is based on *Drosophila* that form was chosen because of the definiteness of the facts and ease of presentation. Goldschmidt's studies will be further noted in a later section. It should be understood that the mechanism of normal sex determination, even in flies, is sometimes enormously more complicated (Metz, 1929) than is suggested by the preceding statement.

These, in brief, are the now known cytological or zygotic foundations of sexuality. In sexual reproduction two kinds of germ cells are contributed by one or the other sex, and each kind definitely predisposes—determines under usual conditions—that a particular sex shall develop from its union with a cell from the other sex. The difference in kind is often visible in the sex chromosomes (X and Y), and sex determination normally follows the distribution of X and Y; it is not chromosomes as such, however, but *genes* carried in all chromosomes— autosomes and sex-chromosomes— that exercise this influence upon the origin and development of sexuality. This genetic complex, whether prospectively male or female, contains also a numerous minority of genes predisposed to the formation of the opposite sex; cytological study has thus discovered and provided a basis for bisexuality in the individual. Indeed the cytology of sex determination is now read partly or largely in quantitative terms—a notable advance and change from the stress it formerly seemed to place upon qualitativeness and fixity. The way in which any gene, or group of genes, exercises its influence on sex has not been thus disclosed, and perhaps this question is to be resolved through physiological rather than cytological study. The physiological approach is treated in later sections.

SEXUALITY IN ANIMALS WHICH FAIL TO DEVELOP GERM GLANDS. It has just been shown that the genic composition of the individual is of much significance in accounting for its sexuality. But we may now ask whether this difference in genes is equally significant in the establishment of the characters which usage has placed in the various categories of sexuality—*primary* (testis, ovary), *accessory* (sperm ducts, prostate, seminal vesicle, penis; uterus or oviduct, clitoris) and *secondary* (hairiness, plumage, size and form difference, voice, behavior, etc.). If not equally significant in all, are the genic differences of some significance in all? The essentials of an answer to these and related questions must now be considered and it happens that those rare developmental abnormalities known as gonadless animals supply specially valuable and nearly crucial evidence.

In the case of the insects no one at present questions the close and fairly direct genic control of all primary, accessory and secondary sex characters. In such forms as the crustacea and vertebrata, however, the well known facts that castration (abrupt removal of the developed primary organ), and also gonad or hormone substitution, are able to modify the development of the remaining accessory and secondary characters have led most investigators to suppose that this hormonal influ-

ence is the sole effective agent for the secondary characteristics in these animals. Much effort has therefore been spent in attempting a fine and real distinction between "sex determination" and "sex differentiation." In the whole of insect sexual development no one has succeeded in finding a trace of evidence that gonads or their products influence the development of any sex character—primary, accessory or secondary. Genic or cellular constitution and influence must there be considered as having a fairly direct influence upon sex characters of every category. In view of this elemental fact, and of the obliqueness of the evidence from the hormones on this special point, it can not be assumed that the genes are without influence on the accessory and secondary characters (sex differentiation) of vertebrates, and that these genes are there effective in the development of primary characters (sex determination) only. The facts obtained from some of the cases of gonadless animals permit no other view than that in vertebrates, as in insects, the genic influence extends in one or another attenuated form to the accessory and secondary characteristics; the failure of the gonad to develop in these cases merely provides an unusually favorable opportunity for the genes to demonstrate their effectivensss. If the hormonal control is an assistant, even an over-towering one for certain characters, that is fundamentally and for the present moment a relatively unimportant matter.

Specific cases. Jacquet (1895) described a case in the lizard, *Lacerta*, in which the gonads were entirely wanting though right and left oviducts were present. The latter were found to open normally into the body cavity anteriorly and into the cloaca posteriorly. Cockayne (1916) recorded the case of a gynandromorph moth hybrid which had no gonads. Adlerz (1908) seems to have described two cases of "imperfect lateral gynandromorphous" ants (*Anergates atratulus*) in which there were no gonads, though male sexual ducts were present on the left side. One of the two ants is described as "evidently feeling itself to be a male but was treated by the males in the colony as a female." Of particular interest here is Harrison's (1920) demonstration that all of the female moth hybrids resulting from the cross, *Oporabia dilutata* ♂ X *O. autumnata* ♀, are without gonads.

Poll (1909) described a duck (*Netta rufina*) having the external appearance of a male but with the male characteristics imperfectly developed. When dissected no trace of gonads could be found, and careful microscopic examination of sections of the entire gonad-renal region showed that no trace of gonadal tissue was present. G. Smith and Haig-Thomas (1913) described four cases of healthy pheasant hybrids which were

without gonads; but each had a very small, narrow oviduct on the left side, with its opening into the cloaca occluded, and the other end narrowing down to a fine filament. These four birds showed male characters in the plumage to slightly varying extents. Riddle (1925a) has reported very fully upon 16 cases of pigeons (12 definitely hybrid) without a trace of gonadal tissue discoverable by macroscopic observation aided with a good hand-lens, and also upon a supplementary group of 15 birds in which one gonad only was abnormally absent. These 31 cases were found at autopsy among about 20,000 birds—a large proportion of these being hybrid. Here it was not only possible to show the extent to which both accessory and secondary characters could develop in what appears to be the congenital absence of the gonad, but also to provide a probable explanation of the developmental cause of the occasional absence of gonads in birds. In many of these cases it was practically certain that the absence of gonads was congenital—not a temporary condition nor one attained shortly before examination of the birds. Such agenesis of gonads is considered a consequence of the failure of "all primordial germ cells" (Swift, 1914; Firket, 1914) to reach or enter the anlage of the germinal ridge; thus no part of this ridge receives its usual and necessary stimulus for gonad formation. Despite this agenesis of the gonads some of these birds developed slightly heavier body weight than their sisters, the narrow pubic bones of the male (probably no sperm ducts) and the most complete and emphatic masculine behavior. On the other hand, some of these birds (genetic females?) with no gonads developed infantile oviducts and neuter or feminine behavior. Crew (1927a) has described a gonadless fowl and discussed the problem.

Zawadowsky and Zubina (1928) studied two hybrid pheasants which, on macroscopic observation, showed no gonads. The whole gonad areas were sectioned and a microscopic examination revealed, in a minute nodule on both right and left sides, a structure said to be like that of undifferentiated or embryonic testis. At least one of these birds had an infantile oviduct. Zawadowsky prefers not to consider these as cases of gonad agenesis, but as originally females whose (left) ovaries disappeared; this view was thought tenable from the fact that these birds originally had female plumage and only gradually (within 5 years) assumed male plumage. In view of these interpretations Zawadowsky thinks it is not certain that in Riddle's cases the development of the secondary sex characters had occurred without the presence of gonads in an early period. On this point the reviewer may here briefly note that the presence in early life of a really good gonad could not account

for the extreme masculine behavior observed in two of these gonadless "fighting males" who were known to have fought for mates till the very date of autopsy—at the age of more than ten years.

Since the differentiation and maintenance of sex characters apart from gonadal influence is here the important point, the *changes* or new differentiations which follow complete castration also belong in this picture; such cases are well known and many specific examples are cited in a later section. Gynandromorphic higher vertebrates afford further special evidence. In these cases certain segments of the animal's body are male and others female, and the atypical distribution of genes or chromosomes provides the basis for this segmental sex difference; Doncaster (1914) has described various methods by which this condition may arise. Dissimilar plumage on right and left halves of the body, male on one and female on the other, characterized the finches of Weber (1890) and Poll (1909) and the pheasant of Bond (1913); Macklin's (1923) fowl showed in addition very unequal size or stature in the right and left sides of the body. Though the germ glands of most of these individuals were also hermaphrodite their secretions were evidently not the decisive factor for the very divergent sexuality expressed on the right and left sides (or various segments) of the body. Since the same sex hormones unquestionably circulate in areas of the two types it is evident that the really effective agent of differentiation is again the chromosomes or genes. From this survey of conditions, under castration, gynandromorphism and gonadless animals, we learn that both accessory and secondary sex characters may arise in the insects, and in all probability also in the vertebrates, without the assistance of an incretion from the gonads.

THE ALLEGED ASEXUAL OR EQUIPOTENTIAL SOMA. At this point we are well prepared to consider the question of the "asexuality," "neutrality" or "equipotentiality" of the embryonic soma. During the past twenty years many competent investigators of sex problems have used these terms. It is true indeed that sometimes these terms have been used rather guardedly—merely to denote a (seemingly) common type assumed by the sexes following castration or ovariectomy—but in view of the preceding discussion it would seem that this terminology should entirely disappear from discussions of any phase of the sex problem. The term "indifferent," in the sense of "not yet plainly differentiated" is undoubtedly useful in descriptive embryology and histology of the early stadia of the gonad.

An "asexual" type of human and certain mammals was described with

qualifications by Tandler and co-workers (1910, 1913). In earlier years, and to some extent, both Biedl (1913) and Steinach (1916) accepted the view that the soma is asexual. The same term and conception was adopted by Lipschütz (1917, 1924) who has urged that in birds and mammals the male and female sex characters begin to differentiate only after the differentiation of the hormone-producing sex gland has taken place. Aron (1922) states that the sexes in Triton are "identical" except in their gonads. For the same animal Champy (1922) writes that "as long as I am unable to distinguish the sex in the embryo I regard it as having no sex." Pézard (1915, 1918) and Caridroit (1922) find a "forme neutre" in castrated birds. Zawadowsky (1922, 1925, 1929) has repeatedly stressed the "equipotentiality" of the soma of both birds and mammals, but has himself clearly stated the facts opposed to the idea of absolute identity of male and female tissues. Finlay (1925) castrated and ovariectomized fowls at an earlier age than did any of the above-named workers and, after a study of the kind and degree of change involved, concludes definitely against the equipotentiality of the sexes in birds. Harms (1922) takes the unusual view that fertilization determines only the gonad, all other sex characters being indifferent and bisexual; also that sex characters may partially differentiate in the absence of a gonad, but in the presence of the gland they differentiate only after, and under the influence of, the gonad. As already indicated above, and as has already been pointed out by several observers, present knowledge of the zygotic mechanism of sex determination is definitely opposed to any real or strict equipotentiality of male and female somas.

THE LABILITY OF SEX AND SOME BASES OF PHYSIOLOGICAL SEX THEORY. The preceding sections are concerned exclusively with the rôle of chromosomes and genes in any and all aspects of sex development. These genetic, constitutional or cytosexual influences have been shown to be—under normal conditions—definite, widespread among organisms, and far-reaching and inclusive within the individual. In the present and later sections it remains to consider some of the facts which show that—under stress, or particular forms of stress—this genic or chromosomal influence on sexuality can be completely overriden or reversed. The basis of sexuality is thus revealed as a labile and quantitative thing with which, perhaps in its entirety, physiology may hope to deal.

Experience has shown that sex development is not equally easily disturbed, reversed or partially reversed, in all classes or phyla of animals. Vertebrates—particularly amphibia and birds—mollusks and some worms have proved of extraordinary interest, since all these groups

have repeatedly supplied cases of complete sex-reversal, and because the conditions or methods associated with the reversal lend themselves to physiological interpretation. The insects have shown a remarkable resistance to modification by the methods of the physiologist or the experimental embryologist; most of the few observed modifications are the result of parasitization, or of hybridization. To the geneticist insects have proved themselves superlative material for deciding the relationship which one or another combination of chromosomes or genes bears to one or another end-result on sex and sexual characteristics; but great as is this contribution it leaves essentially unbridged (except by hypotheses) the enormous gap between the genes and the finished characteristics. In other words, this gap unbridged—except for the slender threads of genic continuity—extends across practically the whole of development. This statement indeed applies to the actual status of our knowledge concerning the (hereditary) basis of any characteristic whatsoever; and some investigators (Riddle, 1917b; Goldschmidt, 1920; Crew, 1927b) suggest that, because of what we have learned of the versatility and reversibility of sex development, we perhaps now have a better grasp of the developmental physiology of sexuality than of any other group of characteristics. For this reason the reviewer considers it specially desirable to outline the essential facts concerning the plasticity of sex, and in this and a following section, to obtain from these cases their special contribution to the physiological nature and basis of sex development.

The evidence for the labile nature of sexuality is of different kinds and of unequal theoretic value; it requires presentation under different headings. Obviously, the evidence from cases of the complete reversibility of sex is of special importance, not only because it alone demonstrates a complete lability of sex but because it contributes greatly to a correct interpretation of such things as hermaphroditism, partial sex-reversal, and the results of castration-substitution studies.

The complete reversibility of sex. If these paragraphs had been written eight years ago only cases in pigeons (Whitman, 1919; Riddle, 1912, 1916, 1917a); amphibia (Hertwig, 1906, 1912; Kuschakewitsch, 1910; King, 1912; Witschi, 1914, 1921; Crew, 1921) and the worm, *Bonellia* (Baltzer 1914) would have required consideration. Now, however, the cases are rather too numerous to consider in this review. In no better way could the present activity in sex research be indicated. At this point the above-mentioned cases of sex-reversal will be considered only in a most general way, since most of them must later be discussed spe-

cifically in connection with the development of sex theory. One or two things require mention here. It is quite true that additional proofs of sex-reversal have been supplied for all of the above-mentioned cases since 1922, and that prior to that time very special proofs were demanded because most cytologists and geneticists were quite unprepared to admit either the possibility of true and complete sex-reversal or the quantitative nature of sexuality. Again, it is notable that all those cases were concerned with treatments applied at the very beginning of development—the egg stage (pigeon, frog, toad), or early larval stage (*Bonellia*). The reversals from female-to-male were accomplished by such methods or agencies as the mating of individuals of different genera or families (pigeons); higher temperature (27°C.) or by "overripening" eggs (during which they absorb water) before fertilization (frogs); and by permitting the larva to attach itself to the pharynx of a female (*Bonellia*). Reversals from male-to-female were accomplished through "reproductive overwork" of the mother (pigeons); desiccation of the egg before fertilization (toad); and by preventing the larva from attachment to a female pharynx (*Bonellia*).

Other studies have demonstrated additional cases of sex-reversal by agencies applied at later stages—even in the adult in a few cases. A fowl that was the mother of chickens, and had her (single left) ovary destroyed by tuberculosis, in later years became a cock in appearance and desire; this bird was then given a female mate and became the father of two chickens (Crew, 1923). A ring dove that produced eggs during all the first half of 1914 ceased laying and after some months developed strong and complete masculine behavior—even forcing her male mate to function as the female of the pair. The autopsy, December 1917, showed that a thoroughly walled-off and inactive tuberculosis had earlier completely destroyed her (single left) ovary, and that two well-formed testes were present (Riddle, 1924a). In neither of these two cases is there the slightest reason to suspect anything other than complete and normal femaleness during their earlier egg-laying periods. They were definitely male at the time of autopsy.

Benoit (1923, 1924) supplied the final fact necessary to a demonstration that complete sex-reversal can be accomplished in chicks after hatching, by surgical removal of the (single) left ovary. Though he did not himself interpret his results as true sex-reversal this interpretation was soon supplied by Riddle (1924b; 1925b) and Greenwood (1925) who showed that Benoit's conception of the right gonad as cytosexually a rudimentary testicle is inadmissible. The basis of success here is that

the right ovary of this species of bird usually, but by no means always, *differentiates* only slightly as ovary before its development is checked and atrophy sets in. The removal of the growing left ovary again calls forth a growth in whatever remaining tissue is capable of gonad-formation. The slightly differentiated right gland can of course form such tissue, and under these special circumstances—which are only imperfectly known—it usually or often develops as a testis; other tissue adjoining the site of the removed left ovary may also develop, and this development too sometimes takes the form and function of testis. Sperm ducts arise also in connection with these new testicular formations. Benoit was able to prove that spermatozoa form in these (sex-reversed) right gonads, and that the transformed females develop the habitus and behavior of the cock.

Prior to Benoit's studies this external masculinization, and some unusual appearances in the right gonad of ovariectomized fowls, had been noted by Goodale (1916), Pézard (1922a) and Zawadowsky (1922). These observers, however, were unable to declare that these right gonads were of truly testicular nature. Further studies on this topic have been made by Domm (1924, 1927, 1929), Finlay (1925), Greenwood (1925) and Zawadowsky (1926, 1928). The several workers seem to agree that these right gonads, as well as many testis-like gonads which may develop on the left, call forth the head furnishings of the male and a certain degree of masculine behavior; Domm (1927) further suggests that these new gonads inhibit the development of male plumage, and probably also the complete development of spurs.

Contributing to this evidence for the reversibility of sex in the fowl is the fact that embryonic testicular tissue develops within ovarian grafts in the fowl, and also within regenerating portions of ovary left in place following partial ovariectomy (Caridroit and Pézard, 1925; Greenwood, 1925; Benoit, 1926, 1929). It is now probably apparent to all that true sex-reversal—spermatozoa from cells cytosexually female—is involved in these cases. In a consideration of these right gonad growths following ovariectomy, and of Domm's work particularly, Lillie (1927) was unwilling to admit these as instances of true sex-reversal; but beginning with 1929 Domm has thus interpreted his own results.

Many additional instances and types of sex-reversal, in phases later than the egg-stage, have been found among amphibians. These have resulted from various procedures. Harms (1922) and Ponse (1924, 1927a, b) have shown that castration of the male or female toad, *Bufo*

vulgaris, is followed by the development of Bidder's organ into an ovary; and Ponse found that the ova produced in this organ develop into apparently normal tadpoles (more than 400 from one such "male"). Bidder's organ is of course an unusual structure, and its cytologic status is certainly not fully known, but the fact remains that Ponse has shown that simple removal of the testes permits it to *complete* its development as an ovary. That it normally develops *partially* as an ovary in this form, without special procedures, is probably only another evidence of the fluidity of sex which is becoming so evident in amphibia generally. This seems much more probable, as the case now stands, than that in the male the cells which lead to Bidder formations have been able to substitute XX for their XY constitution (or another unbalance of genes). Bearing upon this point, as well as upon the whole question of sex-reversal, is the demonstration that oöcytes have been shown to develop within grafted or transplanted testicle of the frog (Meyns, 1912), the toad (Guyenot and Ponse, 1923, 1927; Ponse, 1924), and salamander (du Bois and de Beaumont, 1927). Relatively early in her studies Ponse (1924) could show that the primordial cells of the toad testicle can develop in either direction—male or female. Witschi (1923) obtained an hermaphrodite frog which at one time fertilized the eggs of a normal female—producing both male and female young. Eggs from this hermaphrodite, fertilized by a normal male, gave only females. This shows that the hermaphrodite was XX (female) in sex-chromosome constitution and that all of its gametes—whether ova or sperm—contained a single X-chromosome. Recently Witschi (1929) has fully shown that female frog larvae (*Rana sylvatica*) are transformed into males by keeping them after the 34th day, at or near the maximum temperature which they can endure permanently, i.e., 32°C.

For a long time there has been reason to think that the oyster changes its sex during its life-span. Recently, Orton (1924, 1927) and Spärck (1924) have supplied data which make it practically certain that in *Ostrea edulis* sex-reversal occurs not once but repeatedly. The visible changes from femaleness to maleness begin (Orton) about $3\frac{1}{2}$ days after eggs are produced, and sperm are liberated within two months if the sea temperature has not become too low. Individuals spawning late in the season may remain in the male phase until the following spring. Those spawning early will, after shedding their sperm, attain a neuter phase; and in some cases a second female phase probably occurs within the same season. Spärck apparently considers temperature changes as responsible for the sex changes; Orton has discussed this question only

in a preliminary manner, considering a metabolic rhythm as its basis, and suggesting that a rhythm in the accumulation of *unusable* products in the tissues is notably involved. This case is of theoretic interest because it clearly shows that such a concept as the "timing mechanism" of Goldschmidt is ruled out. Also since, when fully stated, the interpretations of Spärck and Orton are probably the same in the end. If "unused products" do occur it is probably not their mere presence or abundance that really matters; the type, rate and rhythm of tissue oxidations governing their formation (and disappearance) are the things of more probable significance. And "temperature effects," within the range involved, are probably most intimately concerned in changing the rate of oxidation in the tissues.

Cases of partial sex-reversal, castration, implantation, etc. The immediately preceding summary serves of course to place instances of incomplete sex-reversal in their true perspective; these are now recognizable as movable points along a line extending from one sex to the other—any stage actually found showing therefore only one of several attainable stages. Once their nature is understood incomplete reversals, like complete reversals, may contribute much to an understanding of the processes underlying sex and secondary sex characters; in this group of cases we find mammals and man himself well represented. Many of the changes in secondary characteristics following castration, gonad implantation, or hormone injection, are not to be distinguished in principle from their reversion as noted in the preceding paragraphs. Some of these phenomena may therefore be considered under the present heading.

It is a fact of rather more than historic interest that on this subject we owe an unacknowledged debt to Freud (1905) and his followers (Krafft-Ebing, 1892). When Freud began his work heredity was not only considered the most important agency, but one whose force was thought unbreakable, in the psycho-sexual field and in neuroses. He early pointed to a variety of specific and adventitious events in development which limit or modify the influence of heredity. On this borderline of psychology and medicine these workers arrived at conclusions in harmony with the reversible nature of sexuality long before this conclusion was obtained, or granted, by biology.

From among several cases of partial sex-reversal in the human we select two for mention here. In both examples the secondary sex characters of adult normal women later very closely approached those of man, and, after surgical removal of a masculinizing tumor, again became normally female. Sellheim's (1925) case is that of a woman typically

female, with normal sex-life until the age of 43. Within the following year she became wholly masculine in appearance—a "witch" to the neighboring school children; masculine face—with thick heavy beard, kept shortened with shears; body hair, gait and voice of man; torso masculine, and enlarged penis-like clitoris. At operation a uterine myoma (size of man's head) was removed along with the two ovaries. The right ovary was normal; the left also normal except for the close attachment of a chestnut-sized tumor the anatomical nature of which various pathologists left in doubt; it was said to be either modified testis, suprarenal cortex or corpus luteum. Ten months later the woman was again normally female in appearance except for slight excess of facial hair; body hair, gait, voice, clitoris, etc., were again normal. Holmes (1925) in the same year described a rather similar case, in which the masculinizing effect could be definitely associated with a tumor of the suprarenal cortex.

Steinach (1910, 1912, 1913) demonstrated the reversibility of the somatic and psychic secondary sexual characters of mammals; he concluded further that their development or their modification and maintenance are under the control of the sexual glands. Ovaries were transplanted into young castrated male guinea pigs and rats. In such males the mammary glands became functional; body weight, size, skeleton, and character of hair gradually assumed the female type; penis, prostate and seminal ducts diminished in size, while the Müllerian duct (tube and uterus) developed; they became psychically female, and were treated as females by normal males. Young castrated females with testis implants suffered involution of female accessory organs and developed many male characteristics—appearance, size, skeleton, hair and Psyche. Since it had already been learned that simple prepuberal castration alone caused essentially or merely the assumption of an infantile or "neuter" form in man (excepting skeleton, Tandler and Gross, 1909, 1910, 1913), sheep (Franz, 1909; Marshall, 1912), ox (Tandler and Keller, 1910), guinea pig (Halban, 1903), rat (Hatai, 1913), and other forms, it was at once evident that the implanted glands were the source of this masculinizing or feminizing influence. These researches inaugurated a new period in the investigation of the relation which the gonad secretions bear to the development of sexuality. Steinach's results have been in large measure confirmed, and of course considerably extended, by the studies of Lipschütz (1917), Moore (1919, 1922), Sand (1923), and others on mammals; corresponding studies on birds, amphibia and some lower forms will be noted later.

Moore (1921) removed one testis in the rat and replaced it by an ovary; each gland could here continue to grow and produce functional gametes. Sand (1923) demonstrated the effects of simultaneous implantation of ovary and testis into an infantile castrated guinea pig, and of implantation of ovaries into testes of rats and guinea pigs, finding that "artificial hermaphroditism" with pronounced bisexuality of the psychosexual characters were produced in association with the synchronous development of both male and female sexual organs. Obviously these observations oppose the conception of an antagonistic action of ovary and testis, or of their incretions.

The bovine free-martin is a naturally occurring, or one may say accidental, case of parabiosis between male and female twins during embryonic life. The essential facts, as found by Tandler and Keller (1911) and Keller and Tandler (1916) are that the free-martin is a modified female—such modification resulting in all but 6 per cent of the cases of twins of unlike sex; there are two corpora lutea, showing the twins are from two eggs; nearly all degrees of bisexuality are represented among the various cases, the Müllerian ducts being most rudimentary and the vasa deferentia most developed when the gonad most resembles a testicle; and, finally, the two chorions are fused, and bear connecting or anastomosing vessels which establish a common blood supply for this male and female co-twin. These studies have been confirmed and somewhat extended by Lillie (1916, 1917, 1923), Keller (1920) and Zietschmann (1920). Each of these workers of course sought an explanation of the modified ovary and other female organs in the internal secretions of the gonads—a field of study then already fairly developed. Lillie at first assumed that there is some sort of natural dominance of the male internal secretion over the female ones; and later, that the male hormones probably are produced sooner in the testis than in the ovary since, according to Bascom (1923), the interstitial cells of the testis appear to differentiate slightly earlier than those of the ovary. The mammalian parabiosis studies to be next described are adverse to this theory; they suggest that more than a male hormone is involved.

Important investigations of the mutual effects of male and female hormones (really of bloods) in heterosexual parabiosis of the white rat were made by Yatsu (1921) and Matsuyama (1921). Two types of unions were made: castrated males to normal females; normal males to normal females. In the latter it was found that the testes remain normal; the ovary, except for more numerous degenerating follicles, is normal and fecund— even after 135 days— and the uterus little modi-

fied (though sometimes hyperplasia of the subserosa). On the contrary, in the female united to a castrate male none of the follicles develop normally, follicular cysts and corpora atretica are abundantly produced; there is growth of interstitials and their admixture with lutein cells; the uterus is nearly destroyed—hydrometra. The testis was not affected by union with a spayed female. The clear fact emerges that in these unions, effected when the rats were 29–90 days old, the ovary and uterus of the female are much more affected when the testis hormone is absent than when it is present. Yatsu suggests that not only may castration effect changes in the other incretory organs of the male, but some or many of those various incretions may be capable of insulting the ovary and uterus of a female.

In Matsuyama's tests it appears that, after several months, the testicle of a male united with a normal female does undergo considerable degenerative change, and that the ovary suffers rather similarly. Here also these effects were exaggerated if the partner were a castrate. He found too that in a union of normal and castrate females the mammary glands of the normal enlarged, while those of the castrate did not (also Goto, 1922). Indeed, in all these studies there is an absence of a masculinization or feminization of the secondary characteristics of the castrate partner. The explanation of this and other points awaits further study. When a rat or mouse castrate female is united parabiotically with a normal female the uterus of the castrate enlarges, thus proving that the female hormone has reached and affected the parabiotic mate (Martins, 1929).

The vast number of hermaphrodites that have been described—human, all vertebrate classes, and several phyla of higher invertebrates—require a word of comment. It has now become fairly clear that the hermaphrodites found among bisexual species have two modes (or should we say, times or degrees?) of origin, and therefore belong (in a certain and limited sense) to two groups. First, some arise as the sequel to an unusual or aberrant genic (chromosomal) equipment. A second and much larger group, as was definitely pointed out by Riddle (1924a), is formed of individuals which are to be regarded as incomplete sex-reversals. In mammals no complete sexual recharacterization can take place after certain alternative and irreversible developments (for external genitalia) have occurred at points in the embryonic life. The hermaphrodites with a genetic basis—even after excluding gynandromorphs— offer a range of conditions that will not be further discussed here (see Crew, 1927b). An important review of the data on hermaph-

roditism, examined from the standpoint of sex-reversal, has just been supplied in German by Berner (1930). Greil (1924) has fully discussed a number of cases of human sex anomalies, concluding that many etiological factors other than endocrine may be involved; his list includes developmental irregularities, infections, intoxications, trauma during intrauterine life, severe nutritional disturbances of the mother, and psychopathic conditions. We may call attention to the possibilities offered by these agencies for effecting prolonged and profound changes in the metabolic rate during infancy and fetal life. A book by Marañon (1929) discusses sex in the human as a quantitative thing, manifesting many intergradations in physical and psychical spheres. A temporary feminoid tendency is apt to appear in boys at puberty, and a viriloid tendency in women at the climacterium; both, he states, are associated with a temporary unbalance of the endocrine system coincident with these sex crises.

Hermaphrodite birds, amphibia, fishes, and various invertebrate groups, have been described in great number; but, even if space permitted, the usual absence of decisive etiological facts renders these cases of little value to the purposes of this review. Among the numerous and more instructive cases in birds, are the relatively large groups described by Brandt (1889); Boring and Pearl (1914), and Fell (1923), in all of which cases of ovo-testis were found, and all groups also included cases suggesting that sex-reversal was in progress; probably all of these reversals were in a female-to-male direction.

A general relationship of the gonads of birds to their secondary sex characters has long been known. Indeed, Berthold's (1849) discovery that the testis of the cock will sustain the masculinity of the animal if transplanted to another body region, was made at a period too early to make an impression upon the physiology of his time. Shattock and Seligman (1904) inaugurated modern studies of this type in the bird, and showed that more than minimal amounts of testicular tissue were necessary to maintain at their full height the secondary characteristics of the cock. Guthrie (1910) demonstrated the capacity of engrafted gonads to persist in fowls. Goodale (1913) found in ducks and fowls that ovariectomy is followed by the assumption of much of the male plumage and partial masculine behavior; in castrated males the male plumage is retained, the comb and wattles are small, the masculine instincts and behavior lost; the male duck also loses the power to develop the summer plumage. Riddle (1914b) showed that something contained in testis or ovary is capable of specifically modifying the sex

behavior of female pigeons. Several pairs of hybrid doves were mated female-to-female, and the "relative masculinity" of each member of each pair was tested by recording the frequency with which it functioned as a male in copulations with its female mate. Data obtained during four previous months served as control, and for orientation of tests on the efficacy of the gonad extracts. Intraperitoneal injections of saline extracts (+ some macerated tissue) of bird ovaries or testes were made on alternate days for one month. When the more "masculine" member of the pair received ovarian extract, and the more "feminine" member got testis extract, the copulatory behavior of the pair was reversed during the period of treatment. In most cases the original sex expressions returned soon after injections were discontinued.

Goodale (1918) transplanted ovaries into castrated cocks and ducks and in one case obtained an ovarian graft in the presence of a testicle. Plumage, comb and wattles were feminized; spurs, behavior and body size were little if at all modified. Pézard (1918) injected an extract of cryptorchid testes of swine into castrated cocks, obtaining prompt and full development of the normal male comb, voice and behavior. Morgan (1919) and Punnett and Bailey (1921) showed that the plumage of hen-feathered Sebright males changes to cock-feathering following castration, and that complete hen-feathering there depends upon two dominant mendelian genes. Morgan thought that in this breed the testis was aberrant in its hormone production, but later studies by Nonidez (1924) and Roxas (1926) indicate this is not true. Roxas showed that the castrated Sebright again reverts to henny plumage when a Brown Leghorn testis is implanted into it, and that a Leghorn capon with a successful Sebright testis graft retains the usual male feathering.

Greenwood (1925) and Willier (1927) have shown that the grafting of testis or ovary upon the early embryo of the chick does not perceptibly affect the course of sexual differentiation even when the graft and host are of opposite sex. Greenwood and Crew (1926) obtained a definite and puzzling result which reflects both the complexity of the problem of plumage as a sexual characteristic of birds, and the inadequacy of our present knowledge. A brown Leghorn hen was ovariectomized when four days old and the testes of a brother implanted. Soon afterward the bird assumed the plumage of a cock, but later moulted into that of hen. Examination revealed that a small fragment of ovarian tissue, then quite degenerate, had not been removed; also an active right gonad of testicular nature had developed on the site of the right ovary, and unusually abundant testicular tissue had developed from the two grafts.

Appel (1929) observed a similar case; and Domm (1927) and others have found that in ovariectomized females bearing no testis transplants this secondary reversion to henny plumage seems rather regularly to occur in birds which survive for periods as long as 6 to 24 months. No really definite explanation for these late-life reversions to female type—even in the presence of large testes—is at hand, but all such cases are apparently contradictory to Goldschmidt's theory of sexuality.

As a result of several recent studies, but chiefly the important work of Finlay (1925), the dependence of the secondary characteristics upon genetic constitution and upon gonadic influences is better analyzed in the brown Leghorn fowl than elsewhere. We therefore present at some length the conclusions which seem to be warranted by this and other work. It is found that unequal amounts of testis tissue condition unequal comb size; this is opposed to Pézard's (1922b) "all or none" law. The testes of the fowl are remarkably stable and seem never to change the course of their development between fairly early embryonic life and the end of life. Ovaries taken from chicks up to 4 days of age and grafted into males (with or without testis) often develop as testicular growths with a testicular endocrine function; older ovaries (from a six-day chick onward) usually or often develop as ovaries when grafted into males. A full explanation of this is not at hand, though of course the older the organ the more complete and definite is its differentiation.

Sex differences which are not conditioned by the gonads are: size, conformation, spurs, the presence of oviduct in females but not in males, voice, and pronounced psychological differences. Some of these are modified by gonad activity, but the fundamental differences can not be obliterated by masculinization or feminization experiments (i.e., by grafts), though practically complete masculinization can occur when the female develops functional testis tissue from her own tissues (e.g., cases of complete reversal in adults observed by Crew and Riddle in hen and dove). Castrated birds of both sexes are of a neutral type as regards plumage and head furnishings; the feathers, though of the general type and color of the cock, are longer and less compact. If testis is present as the sole gonad tissue, both sexes develop perfect cocky plumage and head furnishings; if ovary is the only gonad both males and females develop henny plumage and henny head-furnishings. If both testis and ovary are present in either sex, each gonad has its specific endocrine function, and there is no neutralization of the one by the other. The testis causes a strong, erect, cocky comb to develop in the female as well as in the male; while the ovary tends (partially) to inhibit the

plumage towards the henny type. Here we have not equipotentiality of the sexes, but a definite sexuality of the soma. The gonad only accentuates the differences determined by the zygotic constitution of the individual. The specific determining stimulus is present during embryonic life; later than that, if sex cords are proliferated from the germinal epithelium, even in females, they are prone to differentiate into testis tubules. The ovary of the bird is more unstable than the testis and even after differentiation it may change over and develop testis tissue, with normal testicular endocrine function, if it is subjected to certain abnormal environments.

The accessory genital ducts react to specific hormonic stimulus—Müllerian to ovary, Wolffian to testis. In both male and female the Wolffian ducts and the left Müllerian persist after hatching and both will undergo development even in the same bird if or when their specific stimuli are supplied—and the degree of response indicates a quantitative relationship between hormone and organ; again there is apparently no incompatibility or antagonism of the gonads or their hormones. There is a quantitative relation between testis or ovary on the one hand and the degree of development of certain organs and functions on the other.

Some studies concerned with grafts and secondary characteristics in amphibia may be treated in advance of others concerned with reversal in their germ glands. Stockard (1911) showed that ovarian grafts in the male salamander are possible, and indeed that their most favorable site is within or upon the testis. The early work of Nussbaum (1909) and Meisenheimer (1911) showed that the thumb pads of male frogs disappear after castration, but can again be renewed through testis implantation or injection of testis extract. At the height of the breeding season the male crested *Triton* is elaborately different from the female, and Bresca (1910) proved that within a year after castration most of these male characteristics disappear. Removal of ovaries did not affect the characters of the female. Skin from the back of the female, when transplanted to the back of a normal male (in place of his own comb or crest), develops into the male comb. When pieces of male skin from the side, bearing the characteristic white stripe, are grafted on the side of another male, the stripe remains; but grafted on a female the stripe slowly disappears. The result shows that its presence depends upon the testis.

In several species of amphibia partial sex-reversal has been obtained through parabiosis and by gonad implantation. Witschi (1927) found in male and female frog embryos thus united that the testes were unaffected, but the ovaries of some of the females were transformed to ovo-

testes. In parabiotic larvae of the newt (Witschi and McCurdy, 1929) the ovaries are again much changed towards testicular development, but the testes of the male mate are also notably influenced toward the production of ovarian tissue. Burns (1930) united male and female larvae of *Amblystoma tigrinum* and found that usually the ovaries are partly transformed, but less often the testes undergo the greater degree of transformation. Ingenious transplantations of embryonic gonads or pre-primordia into embryo hosts have been made by Burns (1928) and Humphreys (1929). Both made studies on *Amblystoma* and both obtained evidence of the partial modifiability of both ovary and testis; and this irrespective of whether such gonad is implanted tissue or is that of the host. Swingle (1926) thought it probable that experimental sex-reversal in bull frogs would be limited to the progonad stages of "undifferentiated" races.

The crustacea have provided several cases of interest to sex theory, and two of these require consideration here. Banta (1918) and Banta and Brown (1929, 1930) have shown in the water-flea, *Daphnia*, that external conditions prevalent at a "critical period" with reference to egg-formation decide which chromosomal type is to enter into egg-formation, and therefore into the production of young; in this way and sense a control of sex production is here in the hands of the investigator. Some strains of *Daphnia* also show sex-intergrades, i.e., several secondary characteristics are intermediates of those of the two sexes, and this intergradedness is transmitted to offspring.

The second case is that of the crab, *Inachus*, which undergoes much transformation when internally parasitized by another crustacean, *Sacculina*. G. Smith (1906, 1911) supplied a considerable body of facts concerning this parasitization and formulated a theory concerning it. Part of the body of the parasite projects to the exterior from the abdomen of the crab in which it is living, while root-like processes, avoiding the vital organs, ramify to all parts of the crab's body and absorb nourishment chiefly from its blood. The parasite attacks males and females and causes gonad atrophy and consequent sterility in both. The female is otherwise little affected except that her assumption of adult sex characters is accelerated; the males, however, gradually assume more and more of the very distinctive female characteristics. Many crabs die, but among those not killed the reproductive organs regenerate, females always developing ovaries, and the least injured males, testes; in a fully feminized male, however, a sex gland producing both sperm and eggs is formed—true partial sex-reversal. Smith learned that the blood of the

normal male and female crabs differ greatly, that of the mature female being yellow and rich in fat; that of the normal male is light pink and poor in fat. With Robson (1911) he further learned that under parasitization the blood (and liver) of both the male and immature female had come to have the greater amount of fat and the yellow lipochrome which characterizes the blood of the adult female, and we have seen that this was accompanied by a hastening of the sexual maturity of the female and a *transformation* of the male to approximate femaleness.

In opposition to explanations based on gonad hormones Smith states:

> The facts can be equally well explained on the theory that the testicular cells enter into a chain of metabolic processes in the body which do not pursue their normal course in the absence of the testicular cells. . . . . The reason why *Sacculina* causes the assumption of the adult female state of *Inachus* is found in the facts: (1) that the roots of *Sacculina* elaborate a yolk-substance from the blood of *Inachus* of a similar nature to that which is elaborated in the ovaries of an adult *Inachus;* (2) that in order to elaborate this yolk-substance the roots take up from the blood of *Inachus* the female sexual formative substance, which is the necessary material for forming the yolk; (3) that the female sexual formative substance being absorbed by the *Sacculina* roots is regenerated in excess; (4) that the presence of the female formative substance continually circulating in large quantities in the body-fluids of the infected crabs causes the production of adult female secondary sexual characters, and, when the parasite dies, of yolk-containing eggs.

Obviously there are very many assumptions in this theory, though it should be realized that the lines quoted were written before 1911. It is also clear that this theory bears no kinship whatever with a theory of sex based on differential metabolic, i.e., oxidation, rate. It is above all else a denial that the gonad hormones control the development of secondary sex characters. On the other hand, it will later be made evident that the *facts* of this case conform well to the metabolic theory of sex.

Baltzer (1914, 1926) showed that the larva of the potential male of the worm, *Bonellia viridis*, lives parasitically on the pharynx of the mature female, and that it becomes a functionally mature male only under these conditions. He learned also that this differentiation is in part due to the absorption of specific substances from the tissues of the female, since extracts prepared from either the dried pharynx or intestine will produce nearly the same effect as does the parasitic mode of life. If the potential male fails to obtain attachment to a female pharynx and settles into the mud or sea bottom, it develops as a female. Very similar conditions were found by Gould (1919) to govern the course of sex development in the mollusc, *Crepidula*.

At this point it is necessary to supplement the earlier statement that the secondary sex characters of insects are firmly fixed by genic influence. First, a further word as to the strength of the evidence. The remarkable castration or castration-transplantation experiments that have been carried out (Oudemans, 1899; Kellogg, 1904; Meisenheimer, 1909; Kopec, 1913, Prell, 1915; Hegner, 1914) on insects leave no doubt that the early sex cells (Keimbahn), and the gonad which they form, neither produce nor consume anything which contributes to the development of secondary sex characters of several species of insects. Hegner even destroyed these sex cells in the early embryo, long before the caterpillar stage, and got entirely normal adults except for the absence of the sex glands. But even though the gonads do not govern or modify these secondary characteristics, it is known that there are other conditions which do modify them in certain insects.

Perez (1886) observed that the marked sex dimorphism of the earth-bee, *Andrena*, infected with the little insect, *Stylops*, is modified greatly, each sex becoming somewhat more like the other. Giard (1889) obtained similar results with several species of insects in some of which even the penis was modified as the result of such parasitic castration. Kopec is probably correct in urging that all these changes in insects may rest not upon the removal of a hypothetical secretion of the destroyed gonad, but upon the profound nutritional disturbances involved in the parasitization. We would note that if this is true these cases are probably brought nearer toward explanation by the metabolic theory of sex —since prolonged nutritional disturbance almost necessarily involves change in metabolic rate.

Nachtsheim (1923) has noted that when the female of the Hymenopteran, *Carausius*, is made to undergo development at 25°C. the ventral thorax takes on the coloration characteristic of the male. Of much greater significance is the case of the membracid, *Thelia*, thoroughly studied by Kornhauser (1916, 1919). Early parasitization by *Aphelopus* causes merely a slight reduction of size in the female *Thelia*. In the male, however, it effects many changes, and these are all in the direction of the quite dissimilar female; it assumes the female coloration, female type of wing, size of head, proboscis, legs, intestine and abdomen; it approaches the female by increasing the amount of body fat—a point determined by analyses made for Kornhauser by the reviewer. In these cases the initial effect is not on the testes, since these may be normal, but the changes are associated with a specific type of change in the nymphal metabolism. The normal male nymphs develop and mature

more rapidly, are smaller, store little fat—they are markedly katabolic. The parasitization retards their development, increases size, and adds fat; in these several ways the parasitized male simulates the anabolic female and, though an insect, he assumes many of the secondary sex characters of the female.

Our introductory statement concerning the relation of chromosomes to sex indicated that unusual chromosomal relations in the *Drosophila* zygote may decide the sexuality on an unusual basis. Those unusual relations may also provide intersexes of varying degrees and kinds (Morgan and Bridges, 1919; Sturtevant, 1920; Morgan, 1926) not indicated in that statement. It will be recalled that in *Drosophila* sex determination, in both its normal and aberrant forms, has been interpreted on the basis of a "genic balance."

Hybridization of races or species of moths has been found, particularly by Goldschmidt (1912, 1917, 1925) and Harrison and Doncaster (1914, 1920), to be associated with sex phenomena of considerable interest; the sex ratios are often wholly abnormal, and numerous intersexes of various grades are obtained. It was early made clear that the mere act of crossing is here of real significance in sexuality, and these various authors early agreed that the "chromosomal determiner" of various races has a variable "potency." Goldschmidt (1916, 1920, 1922, 1923) has further elaborated a theory of sex in which this variable potency, and other data from studies on *Lymantria*, are of chief importance. Further reference will be made later to the essentials of this theory which, though involving many assumptions, has consistently recognized the quantitative nature of the basis of sexuality.

Effects of castration on metabolism, endocrines and growth. Parts of the data on those topics are noted in other sections of this paper. Our purpose here is to consider additional studies and, more especially, to emphasize the view that postcastration changes in metabolism, blood character, body growth, functional and size changes in other organs—the glands of internal secretion in particular—are or may be items of extraordinary interest in the sex problem. It is a great defect of most castration studies that these particular aspects of castration have been so little regarded, usually indeed wholly unattended, in favor of such characteristics as horns, beard, spurs, plumage, fins and bristles; these latter are at the fringe of sexuality and may with some right be called "secondary" characteristics, while most of the former are near the center and probably reflect rather well the essentials of sexuality. The literature on castration might suggest the conclusion that every biologist

of this and a previous generation has castrated an animal; but one will thumb many volumes to find even a small group of comparable animals on which one-tenth of the desirable kinds of measurements have been made. It is highly desirable that all necessary kinds of measurement be made on the same material. Any adequate study of the effects of castration in a species would necessarily involve hundreds of individuals, genetically homogeneous, and kept not too closely captive; otherwise, neither the necessary metabolism measurements, erythrocyte and hemoglobin values, growth curves, nor the weights and states of numerous organs, would be accurately measured. Close captivity alone probably produces some of the normal effects of castration, particularly in males; season and temperature comprise another important and complicating factor in any adequate study of the effects of gonadectomy.

Most observers report a lowering of the basal metabolism following castration and ovariectomy. Loewy and Richter (1889), Curatulo and Tarulli (1895), Angolitti (1916), Murlin and Bailey (1915, 1917), Kojima (1917), Heymans (1921), Tsubura (1923), Korenchewsky (1925), Rowe and Lawrence (1928), all report this lowering in moderate degree. The data of Zuntz (1904, 1908) and Lüthje (1902) were not obtained at basal levels. On the other hand, Asher and Baertschi (1920) could find no difference in either sex after gonadectomy. Gräfe (1923) thinks such a lowering occurs only occasionally and with this Du Bois (1924) apparently agrees. In more recent work Lee and van Buskirk (1928) report a decrease of about 10 per cent in a small series of male and female rats. Aude (1927) reports a decrease of 30 per cent in castrated cocks, and found that in these capons the normal metabolism could be restored by injection of extracts of bull or cock testes. Mitchell, Card and Haines (1927) obtained a reduction of 14 per cent in castrated cockerels; they note that the decrease was not immediate in all cases. Slonaker's (1930) measurements of the daily food intake (calories) of gonadectomized and normal rats aged 200 days have an important bearing on this question; the values found were: for the normal males, 101.3, castrate, 55.4; normal females 74.5, ovariectomized, 45.2. Since castration is known to decrease spontaneous activity in many or most animals it is here even more essential than in other metabolism work that this factor of differential activity be rigorously ruled out. If one may believe that this has often or usually been done it is then difficult to avoid the conclusion that gonadectomy moderately reduces the basal metabolism, and also that the reduction is greater in some species than in others. Unfortunately it is still uncertain which sex is the more affected by gonadectomy.

It is well known that gonadectomy usually leads to the attainment of increased body size in many domesticated animals. It is notable, however, that this result has by no means been obtained uniformly in tests made on laboratory animals, and in a sense this result questions the suitability of captive laboratory animals for the study of the real and full effects of castration. Probably, however, the results of Hatai (1915), based on greater numbers and on more homogeneous material, afford an approximately true picture of a few effects of gonadectomy on certain organs of the rat. A definite influence on thyroid weight was not found; suprarenal size was increased in the male and decreased in the female, the hypophyses were much enlarged in the male and slightly so in the female; thymus involution was reduced or delayed in both sexes. A survey of several similar but less notable studies indicates that our knowledge does not extend much beyond these few points.

Extra-gonadal hormones and sex. The previously considered relationships of the gonads to sexuality include of course only some of the relationships of the internal secretions to sexuality. It is possible that other incretions than those of the gonads are closely related even to primary sexuality in those forms that have a highly developed endocrine system.

Castration and gonad implantation involve abrupt insult to an organism, and the modifications of secondary characteristics following castration are not necessarily wholly the result of the loss of testis hormone. Castration and ovariectomy lead to marked changes in other incretory organs, and probably in metabolic rate (Korenchewsky, 1914, 1930; and others), which cannot be left entirely out of consideration. But in view of the recent preparation of relatively pure testis hormone by McGee (1927), Gallagher and Koch (1929) and others, and its proved capacity to correct the castrate condition in mammals and birds, as shown by Moore, Gallagher and Koch (1929), and others, there now remains no doubt that the testis hormone or hormones is specific for the more prominent sequelae of castration. Similarly, the better preparations of the ovarian hormone by Laqueur and associates (1927b), Doisy (1930) and others, have now demonstrated their specific action on accessory and secondary sex characters. In neither case, however, can full and complete replacement throughout ontogeny and life be declared as yet; and in neither case can the unity or singleness of the hormone, as now prepared, be declared. Ultimately, knowledge of the molecular structure of the male and female hormones, and of their proved effect on the metabolic rate, will provide facts of much importance to modern sex theory.

The possible rôle of glands of internal secretion other than gonads in the development of sexuality is at present an intricate problem. In the first place, some of these organs seem to show characteristic size differences in the sexes. Are these then real sex or "secondary" sex characters? If so, how do they arise, and how do they behave under castration? Some of the facts available on these points are considered in other sections. Second, in vertebrates the adequate development of thyroid and suprarenal cortex, and the capacity of ovary and testis to complete their sexual differentiation and to function, all apparently depend upon a hormone or hormones of the anterior lobe of the pituitary (Smith, 1927, 1930). Such indications now receive strong support from a recent study made on a strain of mice in which dwarfism has been shown to behave in inheritance as a recessive mendelian character, depending upon a single gene. Here Smith and MacDowell (1930) find a marked aplasia or under-development of thyroid, cortex, testis and ovary; all of these deficiencies seem to be secondary to the primary failure (hereditary) of development in the anterior pituitary, and all are well restored by daily transplantation of anterior lobes.

The thyroids of vertebrates are intimately and variously connected with sexuality (Hammett, 1923, 1926; B. Zawadowsky, 1927; Gudernatsch, 1930). Hammett found that thyroparathyroidectomy at 75 days in rats only slightly retarded growth in testes and accelerated growth in the male hypophysis; in the female, the ovaries actually lost weight and hypophyseal growth was retarded. Thyroid administration in fowls is reported as acting in a sex-differential manner on color and feather structure in the fowl's plumage (Torrey and Horning, 1925, 1927; Krizenecky, 1926; Kuhn, 1928). The spontaneous activity of the rat is a sex variable which Wang (1923) and Hoskins (1925) showed was enormously decreased by castration and spaying; not merely a loss of gonads, but changes in thyroids, suprarenals and pituitary are almost certainly involved. It is well known that tumors of the suprarenal cortex lead to virilism, or masculine characterisation, and two cases in the human were cited above. Here we face the question whether the cortical cells effect this transformation by secreting the same or a different hormone than that produced by the testis. Again, in at least many of the cases of sex-reversal the reversed gland has proved its ability to secrete the hormone of the new sex type; does this circumstance forecast the molecular similarity (not identity) of the two (alternative) substances which the same germ cell is potentially able to produce? Here are major problems, difficulties and incentives for the future.

Reiprich (1925) and Riddle (1927a) have studied the retarded growth, or fetal involution, of the testes in humans and birds, and conclude that this probably results from the presence of female hormone derived from the mother during (ovarian or) embryonic life; Scammon (1926) reports an accelerated growth of the human uterus after the seventh month from the same cause. Laqueur (1927) and others have described an antimasculinizing action of the ovarian hormone when injected into *young* mammals, and Riddle and Tange (1928) obtained the same result in young pigeons. It is now highly probable that this action was indirect—through depression of the secretion of the anterior pituitary, as noted below. The whole of the sex hormone problem is complicated by reports of female hormone in the blood (Frank and Goldberger, 1928; and others) and urine (Laqueur, et al., 1927a; Loewe, Voss, et al., 1928) of potent males; also by the huge amounts of the female hormone obtainable from the placenta, and by alleged oestrus as the result of injecting testis extracts into spayed females.

Smith (1926) and Zondek and Aschheim (1927) proved that gonad growth and time of sexual maturity are controlled by a hormone of the anterior pituitary, which may here be called the gonad-stimulating hormone. During the last two years that basic fact has been so utilized as to clear up many puzzling phenomena in the field of castration-transplantation-injection study, and to afford a fair view of the dynamics of gonad-pituitary interaction. In fact, before the action of the anterior pituitary hormone was known Hammond and Marshall (1923), in searching for the reason that young animals do not produce germ cells, supplied much evidence that this is because the *development* of the sex glands depends upon substances which are present in the blood *in limited quantities only*. Engle (1927, 1929) showed that when ovary and testis are brought together in a graft there is no antagonism of the two, nor of their hormones, but a *competition* for "gonad stimulating" hormone which he overcame by daily anterior lobe transplantation. Evans and Simpson (1929) found that after castration the pituitaries of either sex have a much greater sex-stimulating capacity when transplanted into young females than do those from normal animals. Again, it was found that both ovary and testis in pigeons (Riddle and Tange, 1928), and ovaries in the monkey (Allen, 1928), are actually reduced in size by the administration of the female hormone (oestrin). Very recently Moore and Price (1930) and Moore (1930), who with collaborators have since confirmed some of the above-mentioned studies, have been led to the further conclusion that the gonad hormones and the gonad-stimulating

hormone of the pituitary *closely control each other.* Injected gonadal hormone of either type—male or female—depresses the release of the gonad-stimulating hormone by the pituitary, and thus the gonad itself (if not castrate) is unsupported and declines (since gonad hormones support accessory and secondary sex characters but not the gonad itself); either an external supply of gonad hormone or a tendency to such excess from the gonad itself, will induce the recession in the amount of gonad-stimulating hormone from the pituitary—which, in turn, will soon reduce the hormone output of the gonad. On the contrary, according to Moore, when the amount of gonad or of its hormone output is small the pituitary then furthers its growth and hormone secretion by supplying an effectively larger amount of gonad-stimulating hormone.

There can be little doubt that the above facts and conceptions from investigations made in the past three years will prove capable of resolving some of the obscurities which have been left as such in the preceding pages.

THE NATURE AND PHYSIOLOGICAL BASIS OF SEX. Two kinds of facts are present and outstanding in all the material of the preceding pages. First, genetic sex differences tend to cling to, and express themselves in, all organs thus far considered; two types of genic (chromosomal) difference usually decide the type of gonadic tissue, and in addition a certain persistent sex difference in all the somatic tissues—including those that display "secondary" sex characteristics. Second, an uninterrupted series of cases shows that under a certain type or types of experimental procedures those genic foundations are not substantial and enduring, but reversible. Within this mixture of stability and flux one witnesses some extraordinary things: In the sex-transformation of an animal we do not get an entirely *new* sex but always the other one of a familiar pair —or one or another step in precisely that direction (occasionally a super-male or super-female). Again, we have seen that many at least of the "secondary" (somatic) characters do not reverse quite completely (after castration, new gonad graft, etc.), because of the persistent influence of the genic difference in those tissues; while on the other hand, it was indicated that the primary sex glands can and do sometimes undergo a complete and thoroughgoing transformation; the type of sex cell and the type of hormone both being changed to the alternative type. We thus obtain the surprising result that in many cases where we have found (and applied very early) the adequate means for sex-reversal, this reversal is accomplished with even greater precision and completeness in the *primary* than in the accessory and secondary characteristics.

A more constructive examination of these and other basic facts of sexuality may now be undertaken. Unless we get beyond a mere genic or chromosomal basis of sexuality—which has completely broken down under the pressure of experimental procedures—we are quite without definite knowledge of the distinctive or intimate nature of sex; and also without a dynamics that will account for the effectiveness of several procedures in swinging sex development in *alternative* ways—toward testis or toward ovary formation. Let us note carefully that it is not at all the origin, the presence, nor the development of a part that is here to be accounted for; but merely, what it is that decides that one or the other of two alternative types of cellular *differentiation* shall occur. Chromosomes and genes remain essentially morphological things, and perhaps it would be surprising if conceptions based directly upon them should have proved adequate vehicles for a sexuality that can become fluid and even defiant of the sex-differentiating power of the chromosomes. A physiological theory, based on physiological measurement of sexuality in both its stable and fluid states, should prove more useful. The essentials of a current and growing conception of this kind are outlined below.

The first observations of importance to the theory that maleness and femaleness rest initially upon sustained differences in oxidation rate were made by Geddes and Thomson (1890). Their data were indeed not closely quantitative, being obtained through looking widely at living things, but they led to the conclusion that males are predominantly katabolic; females, anabolic. "According to this view the deep constitutional difference between the male and the female organism, which makes the one a sperm-producer and of the other an egg-producer, is due to an initial difference in the balance of chemical changes." For them all sperm were male; all ova, female. In apparent opposition to the latter conclusion genetic research later proved that sperms may be either male-producing or female-producing; and ova likewise, male-, or female-producing. Still later, however, Riddle (1912, 1914a, 1916, 1927b) was able to differentiate the two sex types of ova (yolks) of doves on a metabolic basis, finding that here too the female type was one in which the water-content was slightly less, fat and lecithin percentage higher, and total of stored material greater; this high *storage* capacity of the female-producing ovum was interpreted as an index of low *oxidation* rate in this cell. Riddle (1917a–b, 1928) also obtained evidence that these oxidation, or metabolic, differentials persist under those conditions which either modify sex, or effect complete sex-reversal, and thus persist

in their relation to sexuality in cases where the chromosomal relationship to sex breaks down.

Of course, much of the evidence for the conclusions stated above was somewhat indirect, since one and the same fresh ovum cannot be hatched for sexing, and be also chemically analyzed or burned in a bomb calorimeter; but a basis for effective study was provided by studies of Whitman (1919) and Riddle in which, among other things, it was found that a large excess of females results from the eggs obtained from doves forced for months to ovulate excessively and at an abnormally rapid rate. The earlier eggs of such series were more masculine (and this was shown also in other ways than in the sex ratio) than the later ones of such a series. Again, in this material evidence was produced by Whitman and by Riddle (1917a, 1925c) that the mere act of crossing doves or pigeons belonging to different genera or families (even the latter is possible in the Columbae) effects the reversal of sex in some of the female-producing eggs; in this material the evidence indicates that the gonads are perfectly transformed, though a single left oviduct sometimes remains. Nearly or quite all the offspring of "family" crosses are males. The transformation of females to males in these wide crosses is interpreted as the result of an increased oxidizing rate which usually follows the mere mixing of very dissimilar germ-plasms as this is expressed in the "increased vigor" of the hybrid. On this point, and on the basis of his own studies, Castle (1926) concludes "that the zygote is not a mere summation of the factors contained in two gametes, but that the cross-bred state itself is a source of metabolic energy in the zygote."

Data obtained in early studies on sex-reversal in frogs and toads contained no measurements made in terms of oxidation rate but the conditions used make it wholly probable that they require the same interpretation as the pigeon studies. Hertwig (1906, 1912) Kuschakewitsch (1910) showed that forcing frogs' eggs to "overripen" (delay in body cavity, during which they absorb water) before fertilization led to a very high percentage—occasionally 100—of males. Witschi (1914, 1924, 1925, 1929) confirmed these as cases of true sex-reversal, and found further that the reversal in female-to-male direction could be accomplished by forcing the larvae to undergo their development at higher temperatures (27°–32°); and reversals in a male-to-female direction were obtained by the use of quite low temperatures. King (1912) showed that the desiccation of toads' eggs prior to fertilization resulted in 87 per cent of males in an experiment in which the mortality of individuals of unknown sex was less than 7 per cent. Riddle (1914a, 1916)

associated these results from frog and toad with those obtained on pigeons in the following way: The ova of frog and toad (and pigeon) are all water-poor (for living cells) and a higher state of hydration of these colloids should lead to increased cellular oxidations; decreased hydration (toad) to decreased oxidation rate (see Giard, 1894). The sex actually obtained is in agreement in all cases— pigeon, frog, toad—with the indicated direction of change in oxidation rate. Also, in the frog the higher temperature used should provide a sustained increase in the rate of cellular oxidations; while the lower temperature used should markedly depress it. The sex actually obtained is again in agreement with the oxidizing rate in both cases. Thus under three conditions which should lead to increased oxidation rate in pigeon and frog, all lead to maleness (even female-to-male reversal); under three conditions which should sustainedly decrease oxidation rate in pigeon, frog and toad, all lead to femaleness (even male-to-female reversal). These results provided an essentially new, wider and firmer basis for a theory of sex resting on differential oxidation rates—or differential metabolic rate; the word "metabolism" being used here in the rather restricted sense in which it is employed nowadays in the study of heat production or gaseous exchange.

Before proceeding further with an outline of numerous supporting facts let us see whether and how this conception of differential oxidation rates can include the other facts of sexuality established on the basis of genes or chromosomes. According to Riddle the oxidation rate is the really decisive thing, and all the facts are satisfied if it be assumed that the chromosomes or genes exercise their influence on developing sexuality by establishing higher or lower oxidizing rates—thus leading to maleness in the former and femaleness in the latter. But a procedure which acts *specifically* on the oxidizing rate of the ovum or developing organism may override the difference usually fixed by the genes and the sex be thus reversed; this sex-reversal, however, is decided by the same elemental thing (oxidation rate) which decides the sex in all unreversed cases. Though the chromosomal relation to sex fails, sex is established nevertheless by the more fundamental means which the chromosomes normally make use of to establish it.

If one wishes a more concrete picture of the way the entire group of genes in an XY zygote exercise a different influence from those of an XX union, it may readily be obtained by visualizing the chromosomal background. We may suppose that in a certain race or species all the *X*-chromosomes—the two in the female (XX) zygote and the one in the XY (male) zygote—have genes whose effect on oxidation rate is a certain

and similar sum; and that the genes of all the autosomes here likewise have a similar sum of such rates whether they are associated in a cell with XX or XY. Now if the genes of the *Y*-chromosome alone effectively lift oxidizing rates in excess of a lone X, then the union of Y with X will establish a higher total oxidizing rate than that established in the XX combination. XY would give males; XX would produce females. That a gene belonging in autosomes may contribute quite as much, and in quite the same way, as a gene borne in an X or a Y is quite clear; the X or Y usually have much effect because they bear very different numbers of genes, or very different ones. It is not mere theory, but a logical requirement of known facts that not all of the thousands of genes have equal and identical effects on the rate of cellular oxidations; the various genes must be regarded as unlike chemically, they certainly play different and special rôles in growth and differentiation. Where the sex chromosomes provide both unlike genes and unequal numbers of genes to different zygotes it is evident that they constitute a mechanism capable of endowing two types of zygotes with unequal oxidation rates, and thus with unequal and unlike impulses toward sexuality.

In the above statement no quality is attributed to any gene which it can be imagined to be without; and that part of their action which is of significance to sex is a purely quantitative matter, the various genes varying among themselves in the degree of their influence on the oxidation rate of the cell as a whole. No *two* strict "sex" classes of genes, nor the production by them of *two* "sex substances" is assumed; and the same thing involved in normal sex-determination is equally and similarly involved in sex-reversal. The whole system is capable of response to effective, specific and forced changes in oxidation rate that may increase or depress it at any and all life stages—with resulting effects and changes on sexuality corresponding to the earliness, *duration* and intensity of their advent. Against this simple background one may contrast the suggestions of Goldschmidt (1916, 1920, 1923, 1925). Here it was assumed that the genes concerned with sex are enzymes, or bodies of similar nature; that they are of two kinds, that each kind is necessary for the acceleration of a reaction leading to the production of the two sex hormones under which sex differentiation is guided; that these two hormones are being produced (in all body cells of insect types; in gonads of vertebrate types) in unequal amounts; that one of the two kinds must be present in definite excess (the "epistatic minimum") before it produces its results; that the one which starts out in excess, and guides the sexual-

ity expressed in the early differentiating gonad, tends—in cases of intersexuality—to be overcome later ("timing mechanism") by the opposite type.

We may now proceed with the evidence concerning the metabolic basis of sexuality. If sex difference rests primarily on prolonged and sustained rate of cellular oxidations in the egg, and in other *early* life-stages, this difference should also often be well expressed in adult stages; it should usually manifest itself (it is unimportant that in these measurements many individuals of each sex fall definitely into the predominant group of the other sex) in adequate measurements of metabolic rate and in various states or conditions of the blood and tissues which reflect, indicate, or accompany, metabolic rate; and indeed in still other ways it should put itself in evidence. Present knowledge will be briefly sketched from this point of view, but without reference here to the many ancillary cases noted in earlier sections.

Adequate measurements of basal metabolism of the sexes have been made in the young human (Benedict and Talbot, 1921) and fowl (Mitchell and Haines, 1927); in the adult human (Benedict and Emmes, 1915; Gephart and DuBois, 1916), fowl (Mitchell, Card and Haines, 1927), rat (Benedict and MacLeod, 1929) and ring dove (Riddle, Christman and Benedict, 1930). In every case (group averages) the higher metabolism (3+ to 14 per cent) has been found in the male. Very recently, the last-named authors have demonstrated the important fact that in their heat-production the sexes (ring doves) respond differentially to temperature. Measured at 30°C. and 20°C. the values for males were changed by 28.1 per cent; females by only 20.3 per cent. It could be shown that this is a true sex response—the most various racial characteristics leaving the direction (but not the amount) of the response unchanged—and that at the higher temperature the birds are in a non-physiological state in which the male is more insulted than the female.

From survival tests on young embryos of doves and pigeons in high and low oxygen tensions Riddle (1920) got indications of better survival of males at highest pressures and of females at lowest pressures. For the ring dove therefore there is evidence that the male has the higher and the female the lower oxidation rate at all life-stages—egg, embryo and adult.

Rowe (1924) established the interesting fact that the sexes show an intrinsic difference in galactose tolerance, though this difference has not been shown to exist in prepuberal stadia when the tolerance dose is approximately 20 grams in both sexes. The tolerance in the male

remains at this level, but with the onset of the catamenia this advances to 30 in the girl, and soon thereafter to 40 grams. Rowe and Lawrence (1928) find that castration in the male is without effect on galactose tolerance level. In the metabolism of galactose the testes seem to play no part, the ovaries certainly a very notable part. It is well known that in man the sexes exhibit a difference in excreted creatine, and that this substance is probably an endogenous product of metabolism. When about 7 years old the boy ceases to excrete creatine; in the girl this continues until puberty, and thereafter it is found in the urine at various and recurrent periods.

One cannot examine the scattered literature on the relative size of certain organs in the two sexes of vertebrates without inclining to the view that characteristic differences probably exist. Such sex differences, if uniformly present in forms where so much is known of the special physiology of organs, should provide valuable indirect evidence as to the relation of metabolic rate to primary sexuality. Unfortunately a sufficient amount of reliable data for an adequate number of species is not now available. To the reviewer it seems probable that the female has a relatively larger hypophysis and the male the larger "resting" gonad. Further data on the size of several organs in all classes of vertebrates are very desirable.

Because of their relationship to respiration the oxygen carriers of the blood (erythrocytes and hemoglobin) might be expected usually to show a sex difference. Adequate measurements on both erythrocyte number and hemoglobin content have been made on the adult in man (Haden, 1922; Rud, 1923), fowl (Blacker, 1926), common pigeon and ring dove (Riddle and Braucher); in every case (group averages) the higher erythrocyte count (2.3–31 per cent) and the higher hemoglobin value (7–35 per cent) is found in the male. In these data it is further notable that in all cases there is a smaller percentage sex difference in erythrocytes than in hemoglobin (see Riddle, 1930). This seems to indicate that the greater oxygen demands of the male tissues are partly supplied by an increased number of red cells; but in addition, these cells are either larger (improbable) or their hemoglobin is more concentrated. Less adequate data for children and for young fowls, however, have not shown a sex difference. In his study of erythrocyte and hemoglobin values in the fowl Blacker further found that after gonadectomy these values decrease in the male, but do not change in the female; a "feminized" male with one testis replaced by an active ovary assumed the values of a normal female. Antonelli (1914) reported that in castrated dogs the hemoglobin is decreased and the numbers of both red and white cells reduced.

Human blood is well known to show a number of sex differences. Some recent studies indicate that several chemical differences in the blood (and in muscle proteins) are sex characteristics of wide occurrence in animals. Klisiecki (1926) showed that the higher value of urea nitrogen of male human blood is characteristic also of the male blood of several other species, with no exceptions. Tadokoro and associates (1927, 1928) found always higher ash and phosphorus values in female serum globulin and albumin; specific rotatory power of male serum globulin always higher; free amino nitrogen of male serum globulin and albumin always higher; ash and phosphorus of female myosin and myogen always higher; the pH value of these proteins always lower in the female, and their specific rotatory power always higher in the male; and free amino nitrogen of male myosin and myogen always superior.

A lower oxidation intensity seems to be expressed in the average lipoid content of the blood, this being lower in males and higher in females. This relationship appears to hold in normal adults of man (Goettler and Baker, 1916; Bloor, 1916), fowl (Lawrence and Riddle, 1916; Warner and Edmond, 1917); dove (Riddle and Burns, 1927), crab (Smith and Robson, 1911), and probably in the moths studied by Steche (1912) and Geyer (1913). We have already seen that in the sex-reversed male crab, *Inachus*, the blood fat was increased while assuming female characteristics and an ovo-testis; and that the total body fat of the male insect *Thelia* is known to increase coincident with the assumption of female characteristics.

In certain species of protozoa in which sex distinction is very evident, Joyet-Lavergne (1925, 1929) has shown by microchemical tests that the male individuals (cells) contain less lipoid, the females more; indeed, he has supplied as clear evidence for a greater oxidation rate, in the males, and for the primary relation of metabolic rate to sex, as might be obtained from protozoa. Hartmann (1925) has described several instructive cases of "relative sexuality" in protista and in algae, observing that a weak female gamete functioned as a male in association with a strong female gamete, while a weak male functioned as a female in association with a strong male. From this it was concluded that sex in the gamete is a relative state, and that the gamete is sexually bivalent. Working with numerous races, species and genera of fungi Satina and Blakeslee (1926, 1927) have obtained data of much interest. Here the terms male and female are difficult to apply, and instead the signs (+) and (−) are used. The authors state, however, that the so-called (+)

races are probably female, the (−) probably male. The living cultures gave more intense *reduction* of $Na_2T_2O_3$ in the (+) races; and cellular extracts more intense reduction of $KMnO_4$ and in Manoilov tests. These studies thus appear to show the usual relation of sex and oxidation rate. Riddle and Reinhart (1928) supplied evidence that the so-called Manoilov "sex test" is a better test for metabolic level than for sex. Since the test decides which of two samples contains the greater quantity of substances more easily oxidized than is the molecule of dahlia, and since these are mainly storage products found in excess in the female, it follows that the results of studies made with this test on the blood and tissue extracts of numerous plants and animals really suggest a higher oxidation rate in the male and greater storage in the female.

Classifications and categories of sex characteristics tend to lose definiteness and significance when approached from the standpoint of their common origin. But a difference in characteristics does arise from the circumstance that two hormones, not antagonistic but different, are automatically associated with the two types of differentiated gonads; a hormone of course affects special organs or regions, and these two hormones do not affect precisely the same regions, nor always the same region in precisely the same way; the resulting differences may be called secondary sex characteristics. It seems improbable that the primary (somatic) sexuality of an organism can remain unchanged after the brusque removal of gonads fully differentiated and already producing hormone, since this hormone probably was a factor in setting the body tissues at a functional level characteristic for that sex. Much experience is found to support this conclusion and little to question it, and the above statement would probably also apply to a still earlier removal of thyroid or hypophysis. No consideration of the nature of sexuality can hereafter neglect the great amount of chemical and physiological data that bears upon the problem. If the effects of genes or chromosomes on alternative sexuality are now capable of a true interpretation in physiological terms—obtainable from measurement of conditions or factors involved in sex-reversal or on sex in any of its measurable states—then the very center of the sex problem now lies within the field of physiology; a probability that differential oxidation rate is that true interpretation seems to the reviewer to have been established. The laboratories seem to find the same thing that species in nature themselves declare, namely, that the male is characterized by his power to conjugate, the female by her competence to reproduce.

BIBLIOGRAPHY

ADLERZ, G. 1908. Arch. f. Zool., v, 1.
ALLEN, E. 1928. Journ. Morph. and Physiol., xlvi, 479.
ANGOLITTI. 1916. Clin. Veter., xxxix, 195.
ANTONELLI, G. 1914. Policlin., xxi, 97.
APPEL, F. W. 1929. Journ. Exper. Zool., liii, 77.
ARON, M. 1922. Compt. Rend. Soc. Biol., lxxxvii, 248.
ASHER, L. AND W. BAERTSCHI. 1920. Biochem. Zeitschr., cvi, 537.
AUDE, D. 1927. Rev. Fran. d'Endocrin., v, 81.
BALTZER, F. 1914. Mitteil. Zool. Stat. Neap., xxii, 1.
1926. Rev. Suisse Zool., xxxiii, 359.
BANTA, A. M. 1918. Proc. Nat. Acad. Sci., iv, 373.
BANTA, A. M. AND L. A. BROWN. 1929. Proc. Nat. Acad. Sci., xv, 71.
1930. Physiol. Zool., iii, 48.
BASCOM, K. 1923. Amer. Journ. Anat., xxxi, 222.
BENEDICT, F. G. AND L. E. EMMES. 1915. Journ. Biol. Chem., xx, 253.
BENEDICT, F. G. AND G. MACLEOD. 1929. Journ. Nutr., i, 367.
BENEDICT, F. G. AND F. B. TALBOT. 1921. Carnegie Inst. Wash. Publ. 302.
BENOIT, J. 1923. Compt. Rend. Acad. Sci., clxxvii, 1243.
1924. Compt. Rend. Acad. Sci., clxxviii, 1640.
1926. Verh. d. I. Internat. Kong. f. Sexualforsch., Berlin, 18.
1929. Arch. de Zool. Exper. et Gen., lxix, 217.
BERNER, O. 1930. Hirsch's Handb. der Inneren Sekretion, ii, 1143.
BERTHOLD, A. A. 1849. Arch. f. Anat. Physiol. u. wiss. Med., Berlin, ii, 42.
BIEDL, A. 1913. Innere Sekretion, 2nd ed., Berlin-Wien.
BLACKER, L. J. 1926. Biol. Generalis, ii, 435.
BLAKESLEE, A. F. 1922. Amer. Nat., lvi, 16.
BLOOR, W. R. 1916. Journ. Biol. Chem., xxv, 577.
BOND, C. S. 1913. Journ. Genetics, iii, 205.
BORING, A. M. AND R. PEARL. 1918. Journ. Exper. Zool., xxv, 1.
BRANDT, A. 1889. Zeitschr. f. wiss. Zool., xlviii, 101.
BRESCA, G. 1910. Arch. f. Entw.-Mech., xxix, 403.
BRIDGES, C. B. 1916. Genetics, i, 1.
1922. Amer. Nat., lvi, 51.
1925. Amer. Anat. lix, 127.
BURNS, R. K. 1928. Anat. Rec., xxxix, 177.
1930. Journ. Exper. Zool., lv, 123.
CARIDROIT, F. AND A. PEZARD. 1925. Compt. Rend. Acad. Sci., clxxx, 2067.
CASTLE, W. E. 1926. Proc. Nat. Acad. Sci., xii, 16.
CHAMPY, C. 1922. Arch. de. Morph. Gen. et Exper., Fasc. 8, 1.
COCKAYNE, E. A. 1916. Trans. Entom. Soc., London, 243.
CREW, F. A. E. 1921. Journ. Genetics, xi, 141.
1923. Proc. Roy. Soc. B, xcv, 256.
1927a. Proc. World's Poultry Congress, Ottawa.
1927b. The genetics of sexuality in animals. New York—Cambridge.
CURATULO AND TARULLI. 1895. Boll. d. r. Accad. med. di. Roma, xxii, 496.
DAVENPORT, C. B. 1930. Genetics, xv, 401.

Doisy, E. A., S. Thayer and C. D. Veler. 1930. Proc. Soc. Exper. Biol. and Med., xxvii, 417.
Domm, L. V. 1924. Proc. Soc. Exper. Biol. and Med., xxii, 28.
1927. Journ. Exper. Zool., xlviii, 31.
1929. Arch. f. Entw.-Mech., cxix, 171.
Doncaster, L. 1914. The determination of sex. Cambridge.
DuBois, E. F. 1924. Basal metabolism in health and disease. New York.
du Bois, A.-M and J. de Beaumont. 1927. Compt. Rend. Soc. Biol., xcvii, 1323.
Engle, E. T. 1927. Proc. Soc. Exper. Biol. and Med., xxv, 83.
1929. Amer. Journ. Anat., xliv, 121.
Evans, H. M. and M. E. Simpson. 1929. Amer. Journ. Physiol., lxxxix, 371.
Fell, H. B. 1923. Brit. Journ. Exper. Biol., i, 97.
Finlay, G. F. 1925. Brit. Journ. Exper. Biol., ii, 439.
Firket, J. 1914. Arch. de Biol., xxix, 203; 1920, xxx, 395.
Frank, R. T. and M. A. Goldberger. 1928. Proc. Soc. Exper. Biol. and Med., xxv, 476.
Franz, K. 1909. Beitr. zu Geburts. u. Gynäk., xiii.
Freud, S. 1905. Abhandlungen zur Sexualtheorie. Vienna and Leipsic.
Gallagher, T. F. and F. C. Koch. 1929. Journ. Biol. Chem., lxxxiv, 495.
Geddes, P. and J. A. Thomson. 1890. The evolution of sex. Humboldt Publ. Co., New York.
Gephart, F. C. and E. F. DuBois. 1916. Arch. Int. Med., xvii, 902.
Giard, A. 1889. Compt. Rend. Acad. Sci., cix, 708.
1894. Compt. Rend. Soc. Biol., xlvi, 497.
Geyer, K. 1913. Zeitschr. wiss. Zool., cv, 349.
Goettler, A. O. and W. Baker. 1916. Jour. Biol. Chem., xxv, 211.
Goldschmidt, R. 1912. Zeitschr. f. indukt. Abstamm, u. Vererb., vii, 1.
1916. Amer. Nat., 1, 705.
1917. Journ. Exper. Zool., xxii, 593.
1920. Mechanismus und Physiologie des Geschlechtsbestimmung, Berlin.
1923. English edition of above book, London.
1922. Zeitschr. f. Indukt. Abstamm. u. Vererb., xxix, 145.
1925. Biol. Zentralbl., xlv, 134.
Goodale, H. D. 1913. Amer. Nat., xlvii, 159.
1916. Carnegie Inst. Wash. Publ. No. 243.
1918. Genetics, iii, 276.
Goto, N. 1922. Arch. f. exper. Path. u. Pharmak., xciv, 124.
Gould, H. N. 1919. Journ. Exper. Zool., xxix, 113.
Gräfe, E. 1923. Ergebn. Physiol., xxi (II), 1.
Greenwood, A. W. 1925. Brit. Journ. Exper. Biol., ii, 165; also, 469.
Greenwood, A. W. and F. A. E. Crew. 1926. Proc. Roy. Soc., B, xcix, 232.
Greil, A. 1924. Zeitschr. f. Konstit., x, 121.
Gudernatsch, J. 1930. Hirsch's Handbuch für inneren Sekretion, ii, 1493.
Guthrie, C. C. 1910. Journ. Exper. Med., xii, 269.
Guyenot, E. 1927. Rev. Suisse Zool., xxxiv, 127.
Guyenot, E. and K. Ponse. 1923. Compt. Rend. Soc. Biol., lxxxix, 4.
Haden, R. L. 1922. Journ. Amer. Med. Assoc., lxxix, 1496.

HALBAN, J. 1903. Arch. f. Gynäk., lxx, 205
HAMMETT, F. S. 1923. Amer. Journ. Anat., xxxii, 53.
1926. Amer. Journ. Physiol., lxxvi, 69.
HAMMOND, J. AND F. H. A. MARSHALL. 1923. Proc. XIth Internat. Physiol. Congr., 137.
HARMS, W. 1922. Fortschr. d. Naturwiss. Forsch., xi, 189.
HARRISON, J. W. H. AND L. DONCASTER. 1914. Journ. Genetics, iii, 229.
HARRISON, J. W. H. 1920. Journ. Genetics, ix, 195.
HARTMANN, M. 1925. Biol. Zentralbl., xlv, 449.
HATAI, S. 1913. Journ. Exper. Zool., xv, 297.
1915. Journ. Exper. Zool., xviii, 1.
HEGNER, R. W. 1914. The germ cell cycle in animals. New York.
HERTWIG, R. 1906. Verh. deutsch. Zool. Gesellsch., xvi, 90.
1912. Biol. Zentralbl., xxxii, 1.
HEYMANS, C. 1921. Journ. de Physiol. et Pathol. Gener., xix, 323.
HOLMES, G. 1925. Quart. Journ. Med., xviii, 143.
HORNING, B. AND H. B. TORREY. 1927. Biol. Bull., liii, 221.
HOSKINS, R. G. 1925. Amer. Journ. Physiol., lxxii, 324.
HUMPHREYS, R. R. 1929. Journ. Exper. Zool., liii, 171.
JACQUET. 1895. Bibl. Anat., iii, Paris.
JOYET-LAVERGNE, R. 1925. Compt. Rend. Acad. Sci., clxxx, 1074.
1929. Compt. Rend. Acad. Sci., clxxxviii, 818.
KELLER, K. 1920. Wiener Tierärztl. Monatsschr., vii, 146.
KELLER, K. AND J. TANDLER. 1916. Wien Tierärtz. Monatsschr., iii, 513.
KELLOGG, V. L. 1904. Journ. Exper. Zool., i, 599.
KING, H. D. 1912. Journ. Exper. Zool., xii, 319.
KLISIECKE, A. 1926. Biochem. Zeitschr., clxxvi, 490.
KOJIMA, M. 1917. Quart. Journ. Exper. Physiol., xi, 354.
KOPEC, S. 1913. Arch. Entw.-Mech., xxxvi, 440.
KORENCHEVSKY, W. G. 1914. Zeitschr. f. Exper. Path. u. Therap., xvi, 68.
1925. Brit. Journ. Exper. Path., vi, 21.
1930. Journ. Path. and Bacteriol., xxxiii, 607.
KORNHAUSER, S. I. 1916. Anat. Rec. xi, 538.
1919. Journ. Morph., xxxii, 531.
v. KRAFFT-EBING, R. 1892. Psycopathic sexualis. Stuttgart.
KRIZENECKY, J. 1926. Arch. f. Entw.-Mech., cvii, 583.
KUHN, O. 1928. Zeitschr. f. Zucht.-Kunde, iii, 62.
KUSCHAKEWITSCH, S. 1910. Festschr. f. R. Hertwig, ii, 61.
LAQUEUR, E. 1927. Klin. Wochenschr., vi, 390.
LAQUEUR, E., E. DINGEMANSE, P. C. HART AND S. E. DE JONGH. 1927a. Klin. Wochenschr., vi, 1859.
LAQUEUR, E. C., P. C. HART AND S. E. DE JONGH. 1927b. Lancet, ccxii, 1126.
LAWRENCE, J. V. AND O. RIDDLE. 1916. Amer. Journ. Physiol., xli, 430.
LEE, M. O. AND E. F. VAN BUSKIRK. 1928. Amer. Journ. Physiol., lxxxiv, 321.
LILLIE, F. R. 1916. Science, xliii, 611.
1917. Journ. Exper. Zool., xxiii, 371.
1923. Biol. Bull., xliv, 47.
1927. Journ. Exper. Zool., xlviii, 175.

LIPSCHÜTZ, A. 1917. Anz. d. Akad. d. Wiss. Wien, No. 10.
1924. The internal secretions of the sex glands. Cambridge.
LOEWE, S., H. E. Voss, F. LANGE AND A. WÄHNER. 1928. Klin. Wochenschr., vii, 1376.
LOEWY, A. AND P. F. RICHTER. 1889. Arch. f. Physiol., Supplement 174.
LÜTHJE, H. 1902. Arch. f. Exper. Path. u. Pharm., xlviii, 184.
MACKLIN, M. T. 1923. Journ. Exper. Zool., xxxviii, 355.
MARAÑON, G. 1929. Los estados intersexuales en la especie humana. Madrid.
MARSHALL, F. H. A. 1912. Proc. Roy. Soc. B, 85, 27.
MARTINS, T. 1929. Instituto Oswaldo Cruz. Supplemento No. 11, 265.
MATSUYAMA, R. 1921. Frankf. Zeitschr. f. Pathol., xxv, 436.
MCCLUNG, C. E. 1902. Biol. Bull., iii, 43.
MCGEE, L. C. 1927. Proc. Inst. Med., Chicago, vi, 242.
MEISENHEIMER, J. 1909. Experimentelle Studien zur Soma- und Geschlechtsdifferenzierung. Jena.
1911. Zool. Anz., xxxviii, 53.
METZ, C. W. 1929. Proc. Nat. Acad. Sci., xv, 339.
MEYNS, R. 1912. Arch. f. mikros. Anat., lxxix, 148.
MITCHELL, H. H., L. E. CARD AND W. T. HAINES. 1927. Journ. Agric. Res., xxiv, 945.
MITCHELL, H. H. AND W. T. HAINES. 1927. Journ. Agric. Res., xxxiv, 927.
MOORE, C. R. 1919. Journ. Exper. Zool., xxviii, 459.
1921. Journ. Exper. Zool., xxxiii, 129.
1922. Biol. Bull., xliii, 285.
1930. Proc. 2nd Intern. Cong. for Sex Research. London (in press).
MOORE, C. R., T. F. GALLAGHER AND F. C. KOCH. 1929. Endocrin., xiii, 367.
MOORE, C. R. AND D. PRICE. 1930. Proc. Soc. Exper. Biol. and Med., xxviii, 38.
MORGAN, T. H. 1919. Carnegie Inst. Publ. No. 285.
1926. The theory of the gene. Yale University Press.
MORGAN, T. H. AND C. B. BRIDGES. 1919. Carnegie Inst. Wash. Publ. No. 278.
MURLIN, J. R. AND H. BAILEY. 1915. Amer. Journ. Obst., lxxi, 526.
1917. Surg., Gyn. and Obst., xxv, 332.
NACHTSHEIM, H. 1923. Zeitschr. f. indukt. u. Abstamm., xxx, 287.
NONIDEZ, J. F. 1924. Amer. Journ. Anat., xxxiv, 359.
NUSSBAUM, M. 1909. Pflüger's Arch., cxxvi, 519.
ORTON, J. H. 1924. Nature, cxiv, 191.
1927. Journ. Marine Biol. Assoc., xiv, 967.
OUDEMANS, J. 1899. Zool. Jahrb., xii, 71.
PÉREZ, J. 1886. Act. Soc. Linn. Bordeaux, xl, 21.
PÉZARD, A. 1915. Compt. Rend. Acad. Sci., clx, 260.
1918. Bull. Biol. Franc. et Belg. (These de Paris).
1922a. Ann. des Sci. Nat. Zool., vi, 83.
1922b. Journ. de Physiol. et Path. Gen., xx, 495.
1922c. Compt. Rend. Acad. Sci., clxxiv, 1573.
PÉZARD, A. AND F. CARIDROIT. 1922. Compt. Rend. Acad. Sci., clxxv, 910.
POLL, H. 1909. Sitzungsb. d. Gesell. nat. Freunde-Berlin, No. 6, 331.

PONSE, K. 1924. Rev. Suisse de Zool., xxxi, 177.
1926. Compt. Rend. Soc. Phys. et Hist. nat. de Geneve, xliii, 19.
1927a. Compt. Rend. Soc. Biol., xcvi, 595.
1927b. Rev. Suisse Zool., xxxiv, 217.
PRELL, H. 1915. Zool. Jahrb., xxxv, 183; 593.
PUNNETT, R. C. AND P. G. BAILEY. 1921. Journ. Genetics, xi, 37.
REIPRICH, W. 1925. Arch. f. Frauenkunde, xi, 349.
RIDDLE, O. 1912. Science, N. S. xxxv, 462.
1914a. Bull. Amer. Acad. Med., xv, 265.
1914b. Science, N. S. xxxix, 440.
1916. Amer. Nat., 1, 385.
1917a. Journ. Washington Acad. Sci., vii, 319.
1917b. Science, N. S. xlvi, 19.
1920. Proc. Soc. Exper. Biol. and Med., xviii, 88.
1924a. Amer. Nat., lviii, 167.
1924b. Proc. Amer. Phil. Soc., lxviii, 152.
1925a. Brit. Journ. Exp. Biol., ii, 211.
1925b. Anat. Rec., xxx, 365.
1925c. Anat. Rec., xxxi, 349.
1927a. Amer. Nat., lxi, 97.
1927b. Verhand. 1st Internat. Kongr. f. Sexualforsch., Berlin, i, 193.
1928. Scientific Month., xxvi, 202.
1930. Proc. 2nd Internat. Congr. for Sex Research, London (in press).
RIDDLE, O. AND F. H. BURNS. 1927. Amer. Journ. Physiol., lxxxi, 711.
RIDDLE, O., G. CHRISTMAN AND F. G. BENEDICT. 1930. Amer. Journ. Physiol., xciv, 111.
RIDDLE, O. AND W. H. REINHART. 1928. Amer. Journ. Physiol., lxxxvii, 517.
RIDDLE, O. AND M. TANGE. 1928. Amer. Journ. Physiol., lxxxvii, 97.
ROWE, A. W. 1924. Arch. Int. Med., xxxiv, 388.
ROWE, A. W. AND C. H. LAWRENCE. 1928. Endocrin., xii, 591.
ROXAS, H. 1926. Journ. Exper. Zool., xlvi, 63.
RUD, S. E. 1923. Acta med. Scand., lvii, 142.
SAND, K. 1923. Journ. Physiol. Path. Gen., xx, 472.
SATINA, S. AND A. F. BLAKESLEE. 1926. Proc. Nat. Acad. Sci., xii, 191.
1927. Proc. Nat. Acad. Sci., xiii, 115.
SCAMMON, R. E. 1926. Proc. Soc. Exper. Biol. and Med., xxiii, 687.
SELLHEIM, H. 1925. Zeitschr. f. Mikro.-anatom. Forsch.
SHATTOCK, S. G. AND G. SELIGMAN. 1904. Proc. Royal Soc. London, lxxiii, 49
SLONAKER, J. R. 1930. Amer. Journ. Physiol., xciii, 307.
SMITH, G. 1906. Faun. Flor. d. Golf. Neapel. Monog. No. 29.
1911. Quart. Journ. Micr. Sci., lvii, 251.
SMITH, G. AND R. HAIG-THOMAS. 1913. Journ. Genetics, iii, 39.
SMITH, P. E. 1926. Proc. Soc. Exper. Biol. and Med., xxiv, 131.
1927. Journ. Amer. Med. Assoc., lxxxviii, 158.
1930. Amer. Journ. Anat., xlv, 205.
SMITH, P. E. AND E. C. MACDOWELL. 1930. Anat. Rec., xlvi, 249.
SPÄRCK, R. 1924. Rep. Danish Biol. Sta. Copenh., xxx, 1.
STECHE, O. 1912. Zeitschr. f. indukt. Abstamm. u. Vererb., viii, 284.

STEINACH, E. 1910. Zentralbl. f. Physiol., xxiv, No. 13.
1912. Pflüger's Arch., cxliv, 71.
1913. Zentralbl. f. Physiol., B. xxvii, No. 14, 717.
1916. Arch. f. Entw.-Mech., xlii, 307.
STOCKARD, C. R. 1911. Arch. f. Entw.-Mech., xxxii, 297.
STURTEVANT, A. H. 1920. Science, N. S. li, 325.
SWIFT, C. H. 1914. Amer. Journ. Anat., xv, 483.
SWINGLE, W. W. 1926. Jour. Morph. and Physiol., xl, 441.
TADOKORO, T., M. ABE AND S. WATANABE. 1927. Journ. Coll. Agric. Hokkaido Imp. Univ., xix (2): 119.
1928. Journ. Fac. Agr. Hokkaido Imp. Univ., xxiii, 1.
TANDLER, J. 1910. Arch. f. Entw. Mech., xxix, 290.
TANDLER, J. AND S. GROSS. 1909. Arch. f. Entw.-Mech., xxvii, 35.
1913. Die biologischen Grundlagen der sekundären Geschlechtskaractere. Berlin.
TANDLER, J. AND K. KELLER. 1910. Arch. f. Entw.-Mech., xxxi, 289.
1911. Deutsch. Tierärtz. Wochenschr., xix, 148.
TORREY, H. B. AND B. HORNING. 1925. Biol. Bull., xlix, 365.
TSUBURA, S. 1923. Biochem. Zeitschr., clxiii, 248.
WANG, G. H. 1923. Comp. Psychol. Monographs, ii, 1.
WARNER, D. E. AND H. D. EDMONDS. 1917. Journ. Biol. Chem., xxxi, 293.
WEBER, M. 1890. Zool. Anz., xiii, 508.
WHITMAN, C. O. 1919. Posthumous Works, ii, Carnegie Inst. Wash. Publ. No. 257.
WINIWARTER, H. AND K. OGUMA. 1930. Arch. de Biol., xl, 541.
WILLIER, B. 1927. Journ. Exper. Zool., xlvi, 409.
WITSCHI, E. 1914. Arch. Micr. Anat., lxxxvi, 1.
1921. Arch. f. Entw.-Mech. xlix, 316.
1923. Biol. Zentralb., xliii, 83.
1924. Arch. f. Entw.-Mech., cii, 168.
1925. Arch. f. Ver. Sozialanthr. und Rassenhyg., i, 9.
1927. Biol. Bull., lii, 137.
1929. Journ. Exper. Zool., lii, 267.
WITSCHI, E. AND H. M. MCCURDY. 1929. Proc. Soc. Exper. Biol. and Med., xxvi, 655.
YATSU, N. 1921. Anat. Rec., xxi, 217.
ZAWADOWSKY, B. M. 1927. Arch. f. Entw.-Mech. d. Organ., cx, 149.
ZAWADOWSKY, M. M. 1922. Das Geschlecht, etc. (Imprimerie gouvernementale, Moscou).
1925. Biologia generalis, ii, 352.
1926. Arch. f. Entw.-Mech., cviii, 531; 563.
1929. Endokrinol., v, 363.
ZAWADOWSKY, M. M. AND E. M. ZUBINA. 1928. Trans. Lab. Exper. Biol., iv, 175.
ZIETSCHMANN. 1920. Schweiz. Arch. f. Tierheilk., Heft vi.
ZONDEK, B. AND S. ASCHHEIM. 1927. Klin. Wochenschr., vi, 248.
ZUNTZ, L. 1904. Zeitschr. f. Geburt. u. Gynäkol., liii, 352.
1908. Deutsch. Zeitschr. f. Chir., xcv, 250.